Brook Trout abundance and distribution at multiple spatial scales in Lake Superior tributaries

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Abstract

Brook Trout (*Salvelinus fontinalis*) populations and habitat conditions are adversely affected by anthropogenic impacts that could impact abundance and distribution of Brook Trout at different spatial scales. My goal was to examine environmental DNA (eDNA) and underwater video cameras (UWVC) as alternative sampling methods to conventional (electrofishing) methods for measuring abundance and distribution of Brook Trout in stream environments across two sampling years (2019, 2020). My second goal was to use the same alternative sampling methods to examine Brook Trout-habitat associations at three spatial scales and to determine whether the habitat associations are unique to one spatial scale or common among spatial scales. The three spatial scales examined were the segment (>200m), reach (50m) and microhabitat ($1m^2$) scale. Environmental DNA concentrations and UWVC surveys showed a strong agreement with Brook Trout presence/absence (89% and 78%, respectively) and estimated abundance but significant interannual variation existed for both methods between sampling years. Habitat associations determined that Brook Trout are associated with both scale-specific habitat characteristics (i.e., canopy cover at the reach scale, baseflow index at the segment scale) but were also strongly associated with common habitat characteristics (i.e., surface temperature, stream width, watershed size (km^2) and discharge). The results of this study support the use of eDNA and UWVC as alternative methods to electrofishing for determining the presence/absence of Brook Trout and abundance. These results also suggest that both scale-specific habitat variables and habitat variables measured across scales are important factors for Brook Trout abundance and highlights what key habitat characteristics fisheries managers should prioritize for management.

Lay Summary

Brook Trout is a culturally and recreationally important species in Ontario. Brook Trout thrive in cold, clear, highly oxygenated streams and are an indicator of high-water quality. Brook Trout range from the eastern United States to northwestern Ontario but in their southern range, Brook Trout has experienced population declines from climate change, landscape alterations, and the introduction of non-native species that have caused it to seek out areas where habitat is more suitable. Brook Trout populations in Lake Superior tributaries are under-studied due to the vast geographic area of the region, therefore, assessing their movements in relation to habitat conditions across a large landscape is inherently challenging. Electrofishing is a common fish sampling tool in stream environments, but it has the potential to injure or cause mortality to Brook Trout. Electrofishing is labour-intensive, requires several pieces of expensive equipment, and a crew of at least 3 members, making surveying Brook Trout populations difficult. Environmental DNA (eDNA) and underwater video cameras (UWVC) may offer alternative ways to survey Brook Trout populations. One advantage of eDNA and UWVC is that they do not require any handling of Brook Trout and reduce habitat disturbance by removing the need to wade through the entire stream section like you would when electrofishing. Secondly, eDNA and UWVC surveys are relatively easy to deploy, are cheaper, and have minimal equipment requirements, which makes sampling remote stream areas easier. This study showed that alternative, non-destructive sampling tools could be used to survey Brook Trout populations and also be used to examine species – habitat associations at different spatial scales.

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Chapter 1. Introduction

1.1 Background

Freshwater ecosystems are experiencing temperature increases from climate change and landscape disturbance causing rapid declines in freshwater ecosystem biodiversity worldwide (Reid et al., 2015; White et al., 2019). As landscape disturbance and climate change progress, there are significant implications for cold-water aquatic species which may need to shift their distributions to find thermally suitable habitat (Kanno et al., 2011; Isaak et al., 2012). Cold-water habitat losses affect the ability of cold-water species to successfully reproduce and survive as their life-history is directly controlled by temperature (Isaak et al., 2012; White et al., 2019). Additionally, fluctuating temperature regimes could influence the abundance of stream salmonids and drive dispersal toward increasingly lower order, higher elevation streams that provide the coldest habitats in a watershed (Isaak et al., 2012).

Lake Superior tributaries provide habitat for populations of resident Brook Trout (*Salvelinus fontinalis*). Brook Trout thrive in clear, cold, spring-fed water with a preferred stream temperature range of 10-19°C (Waco & Taylor, 2010; Xu et al., 2010). Brook Trout is dependent on groundwater upwelling zones for spawning in the fall, overwintering habitat and thermal refugia during summer months (Curry and Noakes, 1995). During summer baseflow conditions, Brook Trout select localized microhabitat areas of cold water when stream water temperatures approach lethal levels (>25°C) (Baird & Krueger, 2003; Petty et al., 2012). Streams along the northwestern shore of Lake Superior provide suitable habitat for Brook Trout at different life stages as they offer ideal water temperatures, nutrients and food sources, riparian cover and stable flow regimes, all of which are critical

habitat requirements for Brook Trout in streams (Curry and Noakes, 1995; Ballinger et al., 2016).

Brook Trout is imperiled in its native U.S. ranges from anthropogenic landscape disturbances (Hudy et al., 2008; Stranko et al., 2008; Smith et al., 2010). Land-cover changes (Stranko et al., 2008), climate change (Xu et al., 2010), elevated water temperatures (Meisner, 1990), nonnative and exotic species prevalence (Hitt et al., 2017), and habitat fragmentation (Belford and Gould, 1989) have all affected the distribution and abundance of Brook Trout at different spatial scales. The progression of anthropogenic disturbance and climate change may cause Brook Trout to shift their distributions to actively find suitable habitat. However, in northwestern Ontario, Brook Trout populations are not well documented due to the vastness of the boreal forest and the inaccessibility of stream locations in the region; as such, region-specific habitat associations are not well documented. Therefore, the habitat features that Brook Trout require are not well understood in a less disturbed, more pristine region, which could be considerably different than the habitat features influencing Brook Trout in more highly impacted environments. A mutli-scale analysis may offer insights into species-habitat relationships within and among spatial scales. By evaluating Brook Trout- habitat associations at different spatial scales, fisheries managers can maintain the ecological drivers that are critical for Brook Trout distribution in Lake Superior tributaries.

Using a multi-scale approach allows ecologists to identify the importance of abiotic and biotic habitat features on species abundance and distribution at different spatial scales (Poizat and Pont, 1996; Hale et al., 2019). Because the most appropriate scale to study a species is usually unknown, it is best to identify which species–habitat relationships are

strongest using a multi-scale approach (Schneider, 2001; Deschênes and Rodríguez, 2007; Hale et al., 2019). Examining species habitat features at multiple spatial scales may provide a more complete picture of how species respond to habitat changes in stream ecosystems across spatial scales (Hale et al., 2019; Kirk and Wissinger, 2020). Using a multi-scale approach can increase the certainty in species distribution changes as observations are made at both fine and larger scales (Hale et al., 2019; Kirk and Wissinger, 2020). Fisheries managers will benefit from knowing whether Brook Trout-habitat associations are related to a specific spatial scale and if management actions should be implemented uniformly across a broad area or focused specifically at finer spatial scales (Takashina and Baskett, 2015).

Examining populations of stream fish species at different spatial scales requires applying appropriate sampling methods that are consistent and scaled to the area of the survey. When sampling imperiled species, many researchers have shifted from using conventional sampling methods like electrofishing to using underwater video cameras (UWVC) and environmental DNA (eDNA) (Castañeda et al., 2020). Electrofishing is commonly used to measure the relative abundance of fishes in a particular area, however this conventional method may not capture detailed information of species-habitat associations within the sampling area (Snyder, 2003; Frezza et al., 2003). Additionally, electrofishing is time consuming, labour-intensive and may cause injury or stress to fishes (Snyder, 2003). Electrofishing usually samples between 50 m to 100 m of stream habitat in a survey, thus a broader-scale sampling tool would provide information on species distributions across longer sections of stream. Using underwater video cameras can allow researchers to visually inspect the interactions of habitat and individuals of the species of

interest (Frezza et al., 2003). Electrofishing can provide information on the number, species, and size of fish caught in an area, while underwater video cameras allow researchers to observe, at a fine-scale, what microhabitats fish are using in the absence of disturbance influence behaviour (Ebner and Morgan, 2013). Environmental DNA techniques have been used successfully to detect the presence or absence of a target species in large stream environments (Rees et al. 2014; Deiner et al., 2017). Using a combination of methods will likely yield an improved ability to detect Brook Trout in areas that are being surveyed for the first time. Further, data from a combination of methods could be useful for parameterizing models predicting the presence of Brook Trout based on habitat features in the absence of survey data. These methods will also have the ability to refine our knowledge on species- habitat relationships, and potentially provide a useful method of understanding not just presence/absence but also species abundance.

Study Objectives

My goal was to examine the ecological drivers of Brook Trout distribution at multiple spatial scales using alternative sampling methods for assessing Brook Trout abundance and distribution in Lake Superior tributaries. The first objective was to compare the utility and accuracy of these electrofishing, environmental DNA and underwater video camera survey methods to quantify Brook Trout presence/absence and abundance within Lake Superior tributaries. The second objective was to determine which habitat variables are most associated with estimates of Brook Trout abundance measured at three spatial scales (microhabitat, reach and segment) and to determine if species-habitat associations are scale dependent (i.e., unique to a spatial scale) or are similar among different spatial scales.

Study Area

This study took place in tributaries within the watersheds along the northwestern shore of Lake Superior. Lake Superior is the largest freshwater lake in the world by surface area, and contains 10% of the world's freshwater (Ballinger et al., 2016). It has over 4,000 km of shoreline, most of which is entirely covered by forests (85%; Ballinger et al., 2016). Not only does Lake Superior support many industries, including commercial fishing, transportation routes, and tourism, it is culturally significant and supports the needs of local communities (Ballinger et al., 2016). Lake Superior has the coldest surface temperature and mean annual temperature of all the Great Lakes and supports a variety of cold-water aquatic species (Ballinger et al., 2016). Approximately 41% of the water supplied to Lake Superior comes from tributaries which are sourced from groundwater inputs (40-75%) and surface runoff (Ballinger et al., 2016; Grannemann and Van Stempvoort, 2016). The tributaries along the northwestern shore of Lake Superior are considered high-gradient, cold-water environments that contain populations of Brook Trout (*Salvelinus fontinalis*), sculpins (*Cottus* spp.), dace (*Chrosomus* spp.) and introduced salmonids (Ballinger et al., 2016).

The tributaries investigated in this study are all small to medium-sized high-gradient streams that derive their flow from groundwater inputs, surface runoff, or snowmelt (Mucha and Mackereth, 2008; Ballinger et al., 2016). This area is within the Precambrian Shield, thus the topography and soils are variable due to glacial activity, post-glacial melt and river outwash activity (Development and Municipalities 2008; Lakehead Region Conservation Authority 2008; Ballinger et al., 2016). Forest management, timber harvesting, the construction of roads, bridges and culverts, and mining are the main drivers

of landscape disturbance and loss of habitat connectivity in the area. Five quaternary watersheds, thermally classified as cold-water systems, were surveyed in this study: the Mackenzie River watershed (368 km^2); the Pearl River watershed (114 km^2); the Wolf River watershed (736 km^2); the Black Sturgeon watershed and the Coldwater watershed (138km^2; Lakehead Region Conservation Authority 2008; Appendix A-1). This study used data collected by me and the Ontario Ministry of Natural Resources and Forestry (MNRF) at the Centre for Northern Forest Ecosystem Research (CNFER).

Site Selection

Thirty stream segments were selected for eDNA sampling and underwater video surveys: 18 were in the Mackenzie River watershed, 4 in the Black Sturgeon watershed, 3 in the Wolf River watershed, 3 in the Coldwater watershed and 2 in the Pearl River watershed (Appendix A-1). In 2019, 8 reaches were electrofished and in 2020 the same 8 reaches were electrofished with the addition of two reaches (Table 3.1). In total, forty stream segments (treated as independent between the sampling years) were surveyed in this study. The 18 reaches in the Mackenzie River watershed were chosen to conduct electrofishing surveys and all stream segments were selected for underwater video surveys but only 68 microhabitats were used for analysis due to technical errors with the video cameras (Table 3.1; Appendix A-1). Each stream segment contained one reach and two microhabitats.

Stream segments were surveyed at three different spatial scales: microhabitat (1m^2), reach (50m), and broad (>50m). The broad-scale sampling unit were defined as stream segments (>50 m in length), as they are subsections of the drainage network that are relatively homogenous in respect to the physical, chemical, and biological properties

(Fitzpatrick et al., 1998). Stream segments within a watershed were separated by a minimum of 200 m to reduce the chance of capturing and observing the same salmonids between segments. All segments were downstream of natural and man-made migratory barriers (waterfalls and dams) and over 2 km from highways and major roads. At the segment level, environmental DNA was used to determine the presence of and potential relative abundance of a Brook Trout by detecting genetic material (Helbing & Hobbs, 2019; Lacoursière-Roussel et al., 2016; Rees et al., 2014). Environmental DNA was used at this scale as the point where eDNA was collected may have originated upstream (as far as 200 m) and indicates Brook Trout presence in a fairly large area (i.e., segment) (Jane et al., 2015). Within segments, 50 m reaches were selected by identifying flow accumulation pathways within the segment. Once the flow pathway was identified, the 50 m reach began ~2m downstream of the flow accumulation pathway. Electrofishing was used to survey Brook Trout abundance at the reach scale as this is a common conventional survey method to gather relative abundance estimates in stream lengths between 50-100 m (Wildman and Neumann, 2002). Lastly, two underwater video cameras (UWVC) were used to survey microhabitats as they can provide information on fish presence and behaviour at fine scales (Frezza et al., 2003). The downstream video camera was situated adjacent to the inflow of the flow accumulation pathway while the other underwater video camera was situated approximately 25 m upstream of the other camera.

Stream habitat surveys

Standardized habitat surveys were conducted within each 50 m reach. Ten-meter intervals were marked upstream to create 5 transects across the width of the stream. Habitat variables were measured at 5 equidistant points across the transect. At each point, depth

(mm) was measured with a meter stick, substrate temperature (°C) was measured with a Therma Plus Meter and Probe (ThermoWorks, American Fork, Utah), and flow velocity (m/s) was measured with a Marsh 2000 Flo-Mate handheld electromagnetic water flow meter (Hach 2020). At the midpoint of the first transect surface water temperature (°C) was measured with a Therma Plus Meter and Probe (ThermoWorks, American Fork, Utah), and at the midpoint of the first, middle and fifth transect, riparian canopy cover (%) was measured with a densiometer, and stream width was measured using a tape measurer. Canopy cover, stream width and depth, substrate temperature and flow velocity were averaged across all the transects to provide a single reach scale value for these habitat parameters.

Chapter 2: Evaluating environmental DNA and underwater video camera surveys as alternatives to electrofishing for determining Brook Trout abundance

Abstract

Estimating Brook Trout abundance and distribution is inherently challenging with labour-intensive sampling methods like electrofishing, which are difficult to deploy in remote areas. Environmental DNA (eDNA) and underwater video cameras (UWVC) may offer a simpler alternative survey method to identify and potentially quantify Brook Trout populations. This study examined using eDNA and UWVC as an alternative to electrofishing to determine the presence and relative abundance of Brook Trout in 18 northwestern Ontario reaches (8 reaches were surveyed in both 2019 and 2020 plus 2 additional reaches in 2020, creating 18 reaches). Triple-pass electrofishing surveys caught Brook Trout in 15 reaches and did not catch any in 3 reaches. The eDNA samples corroborated the electrofishing presence/absence results for 16 of 18 reaches, and additionally detected Brook Trout DNA in two reaches where Brook Trout were not caught by electrofishing. The UWVC corroborated the electrofishing presence/absence results for 14 of 18 reaches and failed to detect Brook Trout in four reaches where Brook Trout were caught by electrofishing. Although ANCOVA models found significant differences in overall eDNA concentrations and UWVC between sampling years, a common relationship (slope) among years between eDNA and UWVC and estimated Brook Trout abundance was observed, indicating a strong, repeatable association between eDNA and UWVC and estimated Brook Trout abundance. The results of this study support the use of both eDNA and UWVC as alternative methods to electrofishing for determining Brook Trout presence-absence and highlights the potential for these methods to estimate abundance of Brook Trout in streams once the interannual variation can be sufficiently accounted for.

Introduction

Estimating the abundance of fish populations is fundamental to understanding the ecological status of organisms, especially in cases where the target species are imperiled or invasive (Peterson et al., 2004; Chamberland et al., 2014). Sampling methods, which are most often traditional capture-based approaches such as electrofishing, seine netting and gillnetting are commonly used to achieve estimates of abundance (Ebner and Morgan, 2013; Evans et al., 2017). One of the most common and efficient methods for examining relative abundance and biomass of fishes in stream environments is electrofishing (Dalbey et al., 1996; Peterson et al., 2004). Electrofishing allows operators to capture and identify individual fish and methods are commonly available to calculate the abundance or biomass of fish in the sampled reach (Bohlin et al., 1989). When conducted sequentially over time, electrofishing can provide important information like population decline or invasive species introductions, so that appropriate management actions can be taken to mitigate potential impacts (Bohlin et al., 1989).

While commonly employed, electrofishing and other capture-based approaches can be time and labour-intensive, require the effort of multiple individuals in the field and can potentially injure or kill fishes (Dalbey et al., 1996; Evans et al., 2017). Some documented electrofishing-related injuries to fish include severe spinal injuries, internal hemorrhaging, bleeding at the gills, physiological stress, asphyxiation and detrimental effects on embryos (Dalbey et al., 1996; Snyder, 2003). While external injuries are not always obvious, studies have found that spinal injuries occurred in 11% of Brook Trout after intensively electrofishing the same stream for over 6 years (Kocovsky et al., 1997). Further, a review of electrofishing found that over 50% of fish that were internally examined for

electrofishing effects had spinal injuries (Snyder, 2003). Not only does electrofishing cause potential harm to the target species, capture efficiency can be limited by the stream or lake size, sampler bias, size of the species, the type of species and water chemistry of the stream, particularly conductivity (Dalbey et al., 1996; Synder, 2003; Peterson et al., 2004). Because of the potential for significant injury, conducting electrofishing on endangered, imperiled or sensitive species is of major concern for population persistence and alternative methods should be considered whenever possible (Bennett et al., 2016; Ellender et al., 2012).

Recently, passive sampling techniques have gained traction as viable sampling tools to examine the presence, absence or relative abundance of species (Frezza et al., 2003; Carlson and Quinn, 2005; Castañeda et al., 2020). Alternative sampling methods such as underwater video surveys and environmental DNA (eDNA) sampling have been used to assess the status of fish populations, both of which eliminate the adverse handling effects associated with capture-based sampling methods (Rees et al., 2014; Ellender et al., 2012). Environmental DNA (eDNA) has been shown to reliably detect the presence of aquatic organisms using species-specific DNA collected from raw environmental water samples in stream environments (Takahara et al., 2012; Pilliod et al., 2013a; Jane et al., 2015; Laramie et al., 2015; Wilcox et al., 2016; Baldigo et al., 2017; Doi et al., 2017). Similarly, underwater video cameras have been shown to be effective at detecting fish at a range of depths and microhabitats in stream environments with high visibility which may reduce the need for electrofishing surveys (Frezza et al., 2003; Ebner and Morgan, 2013; Castañeda et al., 2020; Hitt et al., 2021). However, in flowing water, previous studies have produced varied results between species abundance and eDNA levels as it is difficult to pinpoint the

origin of DNA and, therefore is commonly used as a qualitative detection tool for confirming the presence-absence of species somewhere upstream of the sampling site in a stream system (Jane et al., 2015; Stoeckle et al., 2017; Laramie et al., 2015). Further, underwater video camera studies tend to focus on examining fish biodiversity and community interaction at small scales, making identification of species, especially those that look and behave similar (i.e., salmonids) inherently difficult (Frezza et al., 2003; Ebner and Morgan, 2013). In northwestern Ontario, Brook Trout is the sole salmonid species and top predator occupying these areas, making it much easier to identify and observe target species when fewer species are present (Frezza et al., 2003; Ebner and Morgan, 2013). These techniques may be useful in northwestern Ontario streams due to their relatively low species richness and difficulty in using conventional sampling methods.

The use of cost-effective and more rapid sampling techniques have the potential to monitor changes in fish populations and distributions more frequently than traditional methods. Environmental DNA sampling can detect genetic material originating from a target species that has been shed into aquatic systems, as opposed to traditional methods that rely on direct contact with target individuals (Rees et al., 2014; Lacoursière-Roussel et al., 2016; Helbing and Hobbs, 2019; Beng and Corlett, 2020). Environmental DNA can originate from feces, saliva, urine, skin cells, gametes or carcasses of an organism that is present the system, thus, eDNA sampling has the potential to detect species in areas where traditional methods may fall short (Rees et al., 2014; Beng and Corlett, 2020; Rourke et al., 2021). The techniques used for sampling eDNA require less sampling effort with an estimated cost 67% less than triple-pass electrofishing (Evans et al., 2017). Sampling eDNA is relatively simple, uses minimal equipment and is non-destructive and non-

invasive to the target species or its habitat (Beng and Corlett, 2020). Several studies have noted high detection probabilities when using eDNA compared to traditional methods for rare and elusive species such as amphibians (Ficetola et al., 2008; Goldberg et al., 2016) and fishes (Jerde et al., 2011; Takahara et al., 2012; Thomsen et al., 2012; Laramie et al., 2015) even when target species occur at low densities (Jerde et al., 2011; Ficetola et al., 2008). Environmental DNA can be analyzed using two different approaches: barcoding and metabarcoding. Barcoding uses a species-specific primer to detect DNA fragments of a single species within the sample while metabarcoding uses universal primers to determine the taxonomic composition within the sample (Beng and Corlett, 2020). Quantitative PCR (qPCR) analyses are commonly used for eDNA barcoding to detect and quantify the relative abundance of DNA sequences from a targeted species (Rees et al. 2014). While metabarcoding can be quantitative, interpreting the results is challenging due to differences in shedding rates and physiological characteristics among species (Takahara et al., 2012; Pilliod et al., 2013b; Doi et al., 2017; Ficetola et al., 2019).

In streams with high visibility (i.e. low turbidity), underwater video cameras (UWVC) can also be used to monitor fish population size, abundance and composition of fish assemblages, as well as observe behaviour without the limitations associated with capture-based techniques (Ebner and Morgan, 2013). Like eDNA methods, underwater camera surveys are cost-effective and can capture ecological interactions in several different habitats without disrupting the site (Carlson and Quinn, 2005; Wilson et al., 2014b; Struthers et al., 2015). Previous studies in stream environments have found variable strengths of correlation between underwater video and traditional sampling methods. For example, a study reported differences in fish community composition when using

underwater video cameras and traditional netting techniques (gillnetting, seine netting; Ebner and Morgan, 2013). In some cases, underwater video cameras were able to identify fish species not captured in nets; however, nets also caught fishes not identified in videos (Ebner and Morgan, 2013). For the endangered Redside dace (*Clinostomus elongatus*) using multiple underwater video cameras was just as effective as electrofishing and seine netting methods for detecting and identifying habitat preferences (Castanaeda et al., 2020). Similarly, a comparison of the use of underwater video cameras to triple pass electrofishing for the detection and identification of habitat preferences for the Eastern Cape Redfin (*Pseudobarbus afer*) found significant and strong correlations between abundance estimates from UWVC's and electrofishing (Ellender et al., 2012).

The current study examined the utility of using alternative sampling methods to assess Brook Trout (*Salvelinus fontinalis*) populations in northwestern Ontario. The vast and rugged terrain of the boreal forest in this region makes sampling Brook Trout populations inherently difficult using electrofishing. Thus, evaluating the correlation between presence/absence and abundance estimates from electrofishing with either eDNA sampling, underwater video analysis, or both, may allow for the use of passive Brook Trout sampling techniques as an alternative to electrofishing in difficult to access, remote stream locations. The objectives of this study were to evaluate the accuracy of, and agreement between electrofishing, eDNA sampling and underwater video surveys to quantify Brook Trout populations and presence/absence within Lake Superior tributary streams.

Methods

Electrofishing, eDNA sampling and underwater video surveys were conducted in 18 reaches in the Mackenzie River watershed; 8 reaches were surveyed in 2019 and the same 8 were surveyed in 2020 plus two additional reaches (Table 3.1; Appendix A-1). Underwater video surveys and eDNA sampling were conducted 1-2 weeks prior to electrofishing surveys as sampling using all methods could not be completed in one day. UWVC surveys and eDNA samples were collected from July 10 to August 5 in 2019 and July 15 to August 2 in 2020. Electrofishing was conducted from August 6 to 28 in 2019 and from August 4 to August 14 in 2020.

Environmental DNA samples were taken immediately upon arrival at the site, before any crew members or equipment entered the stream, to reduce the chance of contamination. Within the same 50 m reach that electrofishing and UWVC surveys were subsequently conducted, triplicate 1-L water samples were collected using sterilized Nalgene bottles along the farthest downstream transect of the stream. Prior to sample collection, Nalgene bottles were placed in 10% bleach solution for 30 minutes, rinsed with double-deionized water and left to dry. Once dried, bottles were paired with their lids and sealed. A Nalgene bottle was filled with only distilled water, labelled as the 'blank' sample and was carried within the cooler to the site to ensure that no contamination occurred from the lab. Once at the site, Nalgene bottles were triple rinsed with stream water to wash away residual double-deionized water from the lab. Bottles were pre-labelled with the site name, sample number and time of collection. The field samples were placed inside the cooler with sterilized ice packs and transported back to the lab for filtration.

Water samples were filtered following the protocols provided by Wilson et al. (2014). Pre- and post-filtration control filters were used for every sample to confirm that filter equipment was not a source of eDNA. While wearing powderless nitrile disposable gloves, filters funnels were placed in 10% bleach solution for 20 minutes between samples and rinsed thoroughly with double deionized water. Two forceps per sample were sterilized with 70% ethanol and lit on fire to burn any residual ethanol to extinction. The sterilized forceps were then used to place Whatman GF/C 1.2 μm pore size filter membranes on the funnel base. Water samples were poured into the funnel (one water sample per funnel) and the EZ-Stream pump (Millipore EZ-Stream) was turned on to allow the water to filter through. Once the 1-L water sample was filtered, a new pair of sterilized forceps was used to roll up and transfer the filter membrane to a labelled, pre-sterilized 15mL sample storage container that contained 1mL of Longmire solution (a lysis buffer that is used for the preservation of eDNA). Lastly, the 'blank' distilled water sample was filtered to make sure that no contamination occurred during transfer. Environmental DNA extraction was conducted using the MoBio PowerWater DNA Isolation kit (MobBio Laboratories, Inc) at Trent University. The BRK2 primer was used for genetic testing of Brook Trout DNA using qPCR (Wilson et al., 2014). Brook Trout environmental DNA levels were recorded as the number of DNA copies per 5 μL. Any DNA level that was less than 0.07 copy per 5 μL was considered a non-detection (Wilson et al. 2014a).

After taking eDNA samples, but prior to UWVC surveys and electrofishing surveys, 15 m block nets were placed perpendicular to the direction of stream flow at the upstream and downstream ends of a 50m reach to prevent fish from escaping the survey area. Underwater video cameras (UWVC) were then deployed to survey Brook Trout

abundance in microhabitats. Within the 50m reach, two Go Pro Hero 5 cameras (GoPro, San Mateo, California) mounted on Manfrotto Compact Light tripods (Manfrotto, Markham, Ontario) were placed in two locations facing upstream. Locations had similar habitat characteristics (i.e., similar substrate, woody debris, depth) and were situated at least 25 m apart from each other. Both cameras were programmed to start recording at the same time and recorded for 1-hour intervals in 1080p linear view after a 15-minute settling period. A metal stake with flagging tape was placed 1 m from the camera lens to delineate the microhabitat sample area which was the camera's field of view up to the stake, an area of $0.94m^2$ (Leitrants 2020; Figure 2.1). Cameras were placed in areas free of visual obstruction from large boulders, downed woody debris and vegetation.

Following UWVC surveys and eDNA sampling, triple-pass electrofishing was conducted within the same blocked off 50 m reach, using a battery powered backpack electrofisher with pulsed direct current (Model LR-24; Smith-Root, Vancouver, Washington). Starting at the downstream block net and moving upstream, horizontal sweeps across the stream width were performed with 2 netters until the entire reach had been covered. All captured fish from each pass were placed in aerated buckets containing ambient stream temperature water. Two more electrofishing passes were conducted using the same methods. The site name, date, water conductivity, start time and shocking seconds were recorded for each of the three passes. Brook Trout from each pass were counted, measured for total length and fork length, and weighed. All other species caught were identified to species, counted, and weighed in bulk. All fish were released back into the stream once the block nets were removed. Electrofishing surveys were used to determine Brook Trout abundance estimates using depletion models (see below).

Underwater video analysis

Underwater video analysis was conducted using VLC media player version 3.0.08. (VideoLan, 2006). The entire 1 h recording period was watched and scored. When an individual fish that could be identified as Brook Trout entered and exited the sampling area, the video time was recorded to determine how long fish spent within the sampling area. The abundance of Brook Trout in the video surveys was summarized by 2 variables: Brook Trout seconds and the maximum number of Brook Trout observed in the sampling area simultaneously (MaxN). Brook Trout seconds was calculated by summing the total time that individual Brook Trout were observed per minute across the 1 h video recording. If 2 or more Brook Trout occupied the sampling area, in a single observation period, then Brook Trout seconds were multiplied by the number of Brook Trout seen in that minute. The maximum number of Brook Trout present per minute in the camera's field of view at the same time was recorded as MaxN. Total Brook Trout seconds was calculated by multiplying MaxN by Brook Trout seconds per minute then summed across all the 1 h recording.

Data Analysis

To estimate Brook Trout abundance, a depletion method known as the "k-pass" removal method was used (Carle and Strub 1978). After each electrofishing "pass" the number of Brook Trout were recorded and were physically removed from the population. Thus, under certain assumptions (i.e., the population of Brook Trout is closed, and the probability of Brook Trout capture (*p)* is constant for all animals and from sample to sample) the overall population size and probability of capture was estimated from the number of Brook Trout successively removed. These assumptions were met in the current

study by closing off the surveyed stream section with block nets and by maintaining consistent sampling effort across electrofishing passes. All analyses were conducted using the statistical software package R (Version 4.2.0; R Core Team 2022) The R package "FSA: Simple Fisheries Stock Assessment Methods" (Ogle et al. 2022) and the *removal()* function that defaults to the Carle and Strub method was used to estimate Brook Trout abundance and capture probability (Carle and Strub 1978).

To examine relationships between electrofishing estimated abundance and eDNA concentrations (i.e., the number of DNA copies per 5µL) and to determine whether eDNA concentrations differed between sampling years, while accounting for the effect of the covariate estimated Brook Trout abundance, I used an analysis of covariance (ANCOVA) after the heterogeneity of slopes were tested to ensure that the slopes were similar between sampling years. To meet assumptions of linearity, histogram plots were generated to visually inspect the distribution of the data, and eDNA values and estimated Brook Trout abundance were $\log_{10}$ transformed. A two-way ANOVA was used to analyze the effect of the sampling year and site on Brook Trout capture probabilities, estimated Brook Trout abundance and eDNA concentrations with no interaction term included. To examine potential differences between Brook Trout weight and length between sampling years, an ANOVA was used to understand if potential differences in DNA copies was due to differences in Brook Trout length and weight between sampling years. Diagnostic plots were generated and visually assessed to ensure residuals were homogenously and normally distributed for all of the above analyses.

To examine relationships between electrofishing estimated abundance, MaxN and BT seconds from underwater video camera surveys, an analysis of covariance (ANCOVA) was

used to determine whether MaxN and Brook Trout seconds significantly differed between sampling years while accounting for the effect of the covariate estimated Brook Trout abundance. Brook Trout seconds and MaxN values were $\log_{10}$ transformed to meet the assumptions of data linearity. Diagnostic plots were evaluated visually to ensure residuals were normally and homogenously distributed. Lastly, a two-way ANOVA was conducted to determine Brook Trout abundance and capture probability differed among sites to understand if the variability among Brook Trout abundance and capture probability is due to the reach sampled.

Results

Triple-pass electrofishing captured 164 Brook Trout in 7 of 8 reaches sampled in 2019 and 134 Brook Trout in 8 of 10 reaches sampled in 2020. Brook Trout were significantly smaller in length in 2019 (mean TL= 71.27 mm, range 36-206 mm) compared to 2020 (mean TL = 80.98 mm, range 29-200 mm; ANOVA $F_{1,296}$=5.05, P<0.05). No significant differences in Brook Trout weight existed between sampling years (mean weight in 2019 = 8.65 grams, range 1-110 grams; mean weight in 2020 = 10.75 grams, range 1 – 106 grams; ANOVA $F_{1,296}$=0.074, P=0.785). Using the Carle and Strub method, the relative abundance of Brook Trout in 2019 and 2020 ranged from 7 to 83 and 2 to 49 individuals, respectively (Table 2.1). There was a significant relationship between the cumulative number of Brook Trout caught and the abundance of Brook Trout estimated from depletion models (Linear regression $F_{1,11}$=558.5, P=<0.001, r^2 =0.98; Figure 2.2). Brook Trout abundance and capture probability differed among sites ($F_{1,9}$= 3.86, P=0.044; $F_{1,9}$=0.16, P=0.031, respectively).

In both years of sampling, Brook Trout eDNA was detected in all 15 reaches where Brook Trout were captured by electrofishing. However, the variability in eDNA concentrations was considerable among sites. For example, in 2020, 39 Brook Trout were captured in the reach Walk10 by electrofishing but eDNA levels were low compared to other reaches where fewer Brook Trout were captured (1.64 copies per 5µL; Table 2.2). Further, eDNA was detected in 2 of 3 reaches where no Brook Trout were captured by electrofishing, but eDNA levels were relatively low in these streams (2.73 and 1.15 copies per 5 µL, respectively). Although variation between eDNA concentrations was found, the sites with low detection values and sites that had DNA detection despite no Brook Trout being caught were used in the ANCOVA analysis. In streams where Brook Trout were not captured, the average number of DNA copies was 1.32 copies per 5µL which was significantly lower than the average of 50.05 copies per 5uL where Brook Trout were captured (ANOVA $F_{1,16}$=5.38, P=0.034; Figure 2.3). Overall, Brook Trout presence/absence collected by electrofishing agreed with Brook Trout eDNA detection in 16 of 18 (89%) streams surveyed.

Environmental DNA concentrations were positively related to Brook Trout abundance estimated from electrofishing over both survey years (Figure 2.4). There was no significant relationship between estimated Brook Trout abundance and the sampling year (ANOVA $F_{3,14}$=16.93, P=0.95) which confirmed the equality of slope of regression lines to meet the assumptions of ANCOVA. Although there was a significant difference in mean eDNA levels between sampling years (ANCOVA $F_{1,15}$= 27.2, P<0.001; Figure 2.3)a common slope between eDNA levels and Brook Trout abundance was found (slope = 0.62 $\log_{10}$ eDNA copies per $\log_{10}$ Brook Trout; $F_{1,15}$=6.92, P<0.001) indicating a consistent

relationship between eDNA levels and Brook Trout abundance in both sampling years, differing only in elevation. Mean eDNA concentrations were much higher in 2020 (adjusted mean eDNA = 70.3 copies per 5µL) than 2019 (adjusted mean eDNA= 6.5 copies per 5µL).

Brook Trout were visually detected with underwater video cameras in 11 of 18 reaches surveyed. In the 7 reaches where Brook Trout were not detected by UWVC, Brook Trout were caught with electrofishing in 4 of the reaches but were not caught in the remaining 3 (Table 2.2). Thus, Brook Trout presence/absence detection by UWVC agreed with 14 of 18 (78%) streams surveyed by electrofishing. There was no significant relationship between estimated Brook Trout abundance and the sampling year (ANOVA $F_{3,14}$=4.39, *P*=0.93) which confirmed the equality of slope of regression lines to meet the assumptions of ANCOVA. There was a significant difference in Brook Trout seconds between sampling years (ANCOVA $F_{1,15}$= 7.051, *P*=0.007; Figure 2.5) while adjusting for the covariate Brook Trout abundance. The results indicate that Brook Trout spent significantly more time within the camera sample area in 2019 (adjusted mean BT seconds = 1573) than 2020 (adjusted mean BT seconds = 484), opposite to the pattern observed with eDNA concentrations. A common slope between Brook Trout seconds and Brook Trout abundance was found (slope = 1.28 $\log_{10}$ Brook Trout seconds per $\log_{10}$ Brook Trout; $F_{1,15}$=12.69, *P*=0.002), indicating a common functional relationship between Brook Trout seconds and Brook Trout abundance in both sampling years (Figure 2.5).

The relationship between the maximum number of Brook Trout seen in the 1 h video (MaxN) and Brook Trout abundance estimates followed the same pattern as Brook Trout seconds. There was no significant relationship between estimated Brook Trout abundance

and the sampling year (ANOVA $F_{3,14}$=7.21, P=0.62) which confirmed the equality of slope of regression lines to meet the assumptions of ANCOVA. There was a significant difference in Brook Trout MaxN between sampling years (ANCOVA $F_{2,15}$= 11.24, P=0.001) while adjusting for the covariate Brook Trout abundance. Like the Brook Trout seconds results, the 2019 sampling year had greater microhabitat abundance (adjusted mean MaxN = -0.55) than 2020 (adjusted mean MaxN= 2.8). A common slope between Brook Trout MaxN and Brook Trout abundance was found (slope = 0.47 $\log_{10}$ Brook Trout MaxN per $\log_{10}$ Brook Trout; $F_{1,15}$=19.4, P<0.001; Figure 2.6).

Brook Trout DNA was detected in all 11 reaches where Brook Trout were observed with UWVC. However, in the 7 reaches where they were not visually detected, 5 streams had detectable eDNA concentrations. The remaining two streams did not detect Brook Trout with UWVC or eDNA. Thus, Brook Trout presence/absence gathered by UWVC videos agreed with Brook Trout eDNA detection in 13 of 18 (72%) streams surveyed.

Discussion

Environmental DNA and underwater video cameras have the potential to be used as tools for detecting Brook Trout in streams in northwestern Ontario. Although variation between sampling years existed in the current study, eDNA results were consistent with electrofishing surveys in determining the presence/absence of Brook Trout in 16 of 18 reaches surveyed. Similarly, although variation between sampling years existed for Brook Trout seconds and MaxN, the underwater video results were consistent with electrofishing surveys in determining the presence/absence of Brook Trout in 11 of 18 reaches. Overall, environmental DNA detected Brook Trout presence/absence more consistently than the underwater video cameras. Further, eDNA concentrations were positively correlated with

Brook Trout abundance estimates from electrofishing. The ability of eDNA to detect Brook Trout and quantify abundance is consistent with previous studies that have found significant relationships between the number of fish captured by electrofishing and DNA levels in stream environments (Thomsen et al., 2012; Pilliod et al., 2013b; Baldigo et al., 2017). I found that Brook Trout were detected using eDNA in streams even when abundance was low. This observation is consistent with past studies that found relationships between fish abundance and eDNA concentrations in streams (Baldigo et al., 2016; Yamamoto et al., 2016; Doi et al., 2017; Levi et al., 2018). However, a fully predictive application of these methods would require an understanding the drivers of the annual variation observed in both UWVC surveys and eDNA samples.

Although eDNA dynamics in lotic environments are not well understood, many studies agree that the rapid degradation time of eDNA (hours to days) make it a useful tool for detecting species as positive detections are likely associated with contemporary presence of species (Pilliod et al., 2013a; Thomsen and Willerslev, 2015). For instance, Sockeye Salmon (*Oncorhynchus nerka*) eDNA varied significantly from day-to-day but eDNA seemed to be conserved within in shorter distances (tens of meters) in running water as opposed to larger distances (>1.5 km), suggesting that eDNA samples collected in this study are likely from Brook Trout currently present within the stream (Tillotson et al., 2018). Another study determined that eDNA was undetectable in lotic environments within 5 days of detecting a target species further suggesting that eDNA collected in this study are likely from the contemporary presence of Brook Trout (Harrison et al., 2019). Overall, the significant relationship and strong agreement between Brook Trout presence/absence, abundance and eDNA reported here indicate that eDNA methods can be used as a suitable

alternative to electrofishing methods for determining the presence or absence of a species and potentially abundance if the causes of interannual variation can be explained.

To be confident that both eDNA concentrations and underwater video surveys provide reliable estimates of Brook Trout abundance, a better understanding of the sources of variation in eDNA levels, Brook Trout seconds and MaxN among sites and between years is required. Although eDNA concentrations were higher in 2020 than 2019, MaxN, Brook Trout seconds and estimated Brook Trout abundance showed the opposite pattern, being lower in 2020. In reaches where Brook Trout were not captured by electrofishing, the average eDNA concentration was 1.32 copies per 5µL and in reaches with Brook Trout captured the average concentration was 50.05 copies per 5 µL. At one site, only 0.27 copies per 5uL were collected while 3 Brook Trout were captured in 2019 while in 2020, 2 Brook Trout were captured and the eDNA levels were 6 times higher at 6.4 copies per 5 uL. Thus, in this study, two Brook Trout was lowest abundance at which eDNA was detected, but repeated samples both spatially and temporally, should be taken to account for variability in eDNA concentrations. Wilcox et al. (2016) found that Brook Trout DNA is generally above detectable thresholds in streams where population densities are greater than 1-2 fish/100m² which suggests that the detection threshold in my study of 50 m reaches could potentially be as low as 1 fish per reach.

Although correlations between eDNA concentrations and Brook Trout abundance estimates were found, high levels of variability suggest these results should be interpreted cautiously. Brook Trout eDNA was detected at 2 sites where no Brook Trout were captured in 2020, and low eDNA levels were measured at a site that had 39 Brook Trout captured in 2019. The general increase in eDNA copy numbers for the same sites in 2020 relative to

2019 was surprising with one site having an 8-fold increase in eDNA copy numbers despite fewer Brook Trout caught than the previous year. The potential for false-positives and false-negatives needs to be considered when interpreting eDNA results, especially in streams where flow can dilute eDNA or move it downstream to areas that target species may not occupy (Curtis et al., 2020). Previous studies have highlighted the variation in eDNA detection sensitivity. Jerde et al. (2011) detected Asian carp eDNA in areas that were previously thought to be uninhabited based on electrofishing. In this study, reaches where eDNA was detected but Brook Trout were not captured by electrofishing could have been false positives. However, due to the nature of stream environments, and although no Brook Trout were captured at sites where eDNA was detected, it does not necessarily mean that no Brook Trout were in the reach, they may have avoided being captured or may be occupying an area directly upstream of the reach. Therefore, quantifying Brook Trout based on eDNA concentrations may result in either an overestimation, underestimation or misinterpretation of the presence of a target species in stream environments, thus, repeated samples across space and time should be taken. Further, applying different sampling efforts (i.e., number of water samples or volume of water) coupled with repeated samples and electrofishing surveys may provide better insight into the variability among sites and samples.

The variation in eDNA concentrations and UWVC surveys I observed between sampling years at the same sites may be due to a variety of biotic and abiotic factors. Higher eDNA concentrations in 2020 may be related to higher stream temperatures that year, increasing the metabolic rate of Brook Trout leading to increased rates of shedding epidermal cells or other secretions (Wilcox et al., 2016; Rourke et al., 2021). The weight of

fish is significantly correlated with the number of eDNA copies produced (Takahara et al., 2012); however, most of the Brook Trout captured in this study were young-of-year fish with a median biomass of 2g which did not differ significantly between years. A large group of small Brook Trout may shed more DNA compared to a single large fish of the same mass, however, eDNA concentrations were higher in 2020 despite capturing fewer Brook Trout. Higher eDNA concentrations in 2020 could be due to higher metabolic rate associated with higher stream temperatures and may not due Brook Trout abundance or biomass differences. Further, the warmer temperatures in 2020 may have resulted in fish using thermal refugia leading to a more clumped distribution within the reach, in theory, if Brook Trout are clumped in small areas, water samples could capture high amounts of DNA before dilution and homogenization of DNA may occur throughout the rest of the reach (Rees et al., 2014; Rourke et al., 2021). Previous studies have found that eDNA concentrations are higher when samples are taken closer to caged organisms, and immediately disappeared when the organism was removed (Pilliod et al., 2013a; Jane et al., 2015). Thus, eDNA samples may have been taken close to an area of "clumped" Brook Trout that are occupying thermal refuge areas in the reach. By considering environmental conditions and the biology of the target species will enhance the reliability of using eDNA to determine abundance levels. .

Underwater video camera surveys found microhabitat occupancy time and MaxN counts were significantly higher in 2019, opposite the pattern of eDNA concentrations. Like eDNA concentrations, Brook Trout occupying microhabitats may have been influenced by abiotic and biotic factors that differed in 2020. Although warmer stream conditions in 2020 may have contributed to an increased metabolic rate in Brook Trout

causing more DNA to shed, it could have also influenced Brook Trout to seek out thermal refugia areas outside of the video survey area (Rourke et al., 2021; Petty et al., 2012). Secondly, cameras were placed in relatively the same location as 2019 (with some deviation in order to place cameras in pools) but streams in 2020 were shallower and had less riparian canopy cover, on average. Shallower streams and less riparian canopy cover may have contributed to the increase in water temperatures causing Brook Trout to move to areas that have stable stream flows, ample riparian cover, and suitable water temperatures, all of which are critical habitat features in which Brook Trout rely on (Raleigh, 1982). Lastly, the deeper streams in 2019 may have caused eDNA to dilute before sample collection resulting in lower eDNA concentrations. However, in 2020, fewer Brook Trout were caught by electrofishing which correlates with the lower occupancy time and overall MaxN counts in 2020. Therefore, the interannual variation between eDNA concentrations may be of greater concern for estimating Brook Trout abundance than the UWVC surveys. Using a combination of methods, and sampling multiple times across seasons may improve our understanding of interannual eDNA and UWVC variation and improve their reliability.

Underwater video detection generally agreed with Brook Trout abundance estimates (78%) and both estimates of Brook Trout abundance at the microhabitat scale (seconds and MaxN) are positively correlated with Brook Trout abundance estimates at the reach scale from electrofishing. However, the UWVC results are quite variable within and among years because fish may be clumped or dispersed depending on habitat conditions and the microhabitats I selected may not have captured this variation. As previously mentioned, differences in MaxN and Brook Trout seconds between sampling years could have been due to changing microhabitat conditions, such as water temperature, depth, and

riparian canopy cover. Streams in 2020 were generally warmer and shallower despite being surveyed at similar times of the year and at the same locations, so it could be likely that Brook Trout sought out deep-pool microhabitats and thermal refugia areas with increased cover in 2020 (Sotiropoulos et al., 2006; Petty et al., 2012). Limited variation between microhabitat conditions existed in this study, suggesting that Brook Trout abundance and occupancy may be influenced by seasonal habitat conditions that fluctuate annually (Petty et al. 2005).

Overall, eDNA concentrations were better correlated to electrofishing abundance estimates than underwater video abundance estimates. This observation is consistent with past studies that concluded that using underwater visual surveys underestimated relative fish abundance (Hitt et al., 2021). However, these studies compared single-pass electrofishing with visual surveys, whereas I compared triple-pass electrofishing which provides a better estimation of Brook Trout abundance (Peterson et al., 2004). Additionally, juvenile Brook Trout often move from deeper pool habitats to shallower riffle habitats to avoid predation from larger Brook Trout that exhibit aggression in thermal refugia areas (White et al., 2019; Hitt et al., 2021). By avoiding predators, juvenile Brook Trout may have also avoided camera detection as cameras were placed in moderately deep-pool areas within the reach. For future studies, increasing the number of underwater cameras deployed and recording in a variety of microhabitat conditions (e.g., pools and riffles) would, in theory, increase the chance of detecting Brook Trout.

Although triple-pass electrofishing can be labour intensive, time consuming, and cumbersome when working in difficult terrain, one of the many advantages of triple-pass electrofishing is that abundance estimates are less affected by differences in catch

efficiency between passes (Peterson et al., 2004). This makes electrofishing a powerful monitoring tool, especially where the probability of catch could vary between reaches over time (Hayes et al. 2007). However, it is important to consider that electrofishing can be biased by site characteristics, fish species and fish size, as larger fish tend to be caught more commonly than small-bodied fish (Peterson et al. 2004). Yet, I captured a wide range of Brook Trout sizes, between 29 mm to 206 mm total length, which weighed as little as 1 gram. Therefore, triple-pass electrofishing in these streams seem sufficient in determining the abundance of Brook Trout no matter their size and distribution within the reach.

A potential alternative to electrofishing is to combine underwater video cameras with eDNA sampling to gain better insights on interannual variation and have more confidence in using alternative methods. Although UWVC did not correlate as well as eDNA detections with Brook Trout abundance, it can be used to confirm the presence of Brook Trout at the reach scale. By exploring the potential of integrating different methods, a variety of questions can be investigated. Underwater videos can provide information on Brook Trout behaviour, habitat usage, species interactions and size classes while eDNA concentrations can indicate approximately how many Brook Trout are occupying the stream. Both methods minimize the potential for harming Brook Trout. However, certain methods are best suited to answer specific questions. For instance, underwater videos are limited by the sampling area and may not accurately estimate the abundance or size of Brook Trout across the whole reach, thus, electrofishing is needed to physically measure and weigh Brook Trout and to estimate Brook Trout abundance. Further, eDNA cannot answer questions related to habitat use, behaviour, and size distribution, thus it is a tool that

should be complemented by a method that has some ability to answer questions related to habitat and fish abundance.

In conclusion, my results suggest that alternative sampling methods could be used to assess distribution and abundance of Brook Trout in northwestern Ontario streams. Environmental DNA samples strongly agreed with electrofishing in determining the presence/absence of Brook Trout and there was a strong relationship between eDNA concentrations and estimates of Brook Trout abundance. Although there was variability in eDNA concentrations between sampling years, a consistent, functional relationship between eDNA concentrations and Brook Trout abundance was found. Similar to the eDNA results, a consistent, functional relationship was also found between Brook Trout seconds and MaxN counts obtained from the underwater video surveys and Brook Trout abundance but the overall correlation between surveys was lower than the eDNA results. Despite underwater cameras underperforming at detecting Brook Trout, I wouldn't rule it out as a tool for assessing fish populations especially with the variability in eDNA concentrations. Increasing the number of underwater video cameras within the reach may improve detection probabilities and be less influenced by patchy distributions of Brook Trout. As methodologies continue to improve, the need for capture-based methods may be reduced, future studies could complement capture-based methods with passive sampling methods to improve on the ability of new techniques to quantify fish populations, especially in stream environments.

Chapter 2 Tables

Table 2.1 Estimated abundance and capture probability of Brook Trout in 50m reaches of 15 sampled streams in 2019 and 2020, using the Carle and Strub method. Only sites where Brook Trout caught are shown.

Site Name	Year Sampled	Number of Brook Trout captured[1]	Estimated number of Brook Trout abundance[2]	Probability of capture
EW1K	2019	83 (56,15,12)	86 (±3.25) (82.75-89.25)	0.63 (±0.06) (0.47-0.69)
YoungIn_Below	2019	7 (2,3,2)	8 (±3.49) (1.16-14.84)	0.41 (±0.31) (-0.19-1.01)
Dsouth	2019	2 (2,1,0)	3 (±0.27) (2.47-3.52)	0.75 (±0.27) (0.23 – 1.27)
Walk6	2019	8 (3,3,2)	9 (±3.05) (3.03-14.97)	0.44 (±0.27) (-0.09-0.98)
EW_17	2019	17 (12,2,3)	17 (±1.03) (14.98-19.01)	0.68 (±0.13) (0.43-0.93)
EW_20	2019	32 (26,4,2)	32 (±0.58) (30.86-33.14)	0.8 (±0.07) (0.66-0.94)
Walk10	2019	39 (23,8,8)	44 (±4.87) (34.46-53.54)	0.5 (±0.11) (0.28-0.72)
EW1K	2020	49 (41,4,4)	49 (±0.70) (47.63-50.37)	0.80 (±0.70) (0.69-0.92)
160719	2020	22 (9,7,6)	30 (±10.7) (8.97-51.03)	0.34 (±0.18) (-0.02-0.70)
YoungIn_Below	2020	15 (9,3,3)	16 (±2.13) (11.83-20.17)	0.56 (±0.17) (0.23-0.88)
Dsouth	2020	2 (2,0,0)	2 (±0.0)	1 (±0.0)
Walk6	2020	10 (6,3,1)	10 (±0.86) (8.31-11.68)	0.67 (±0.17) (0.33-1.00)
EW_17	2020	14 (4,5,5)	23 (±17.7) (-11.77-57.77)	0.25(±0.25) (-0.25-0.75)
EW_20	2020	21 (14,4,3)	21 (±1.16) (18.72-23.28)	0.68(±0.12) (0.45-0.91)
WwalkS1L	2020	4 (3,1,0)	4 (±0.21) (3.6-4.4)	0.8 (±0.21) (0.4-1.2)

1-Values in brackets indicate the number of Brook Trout captured in each electrofishing pass.
2- The estimated number of Brook Trout was determined by the Carle and Strub removal methods, the bracket indicates the standard error, and the following brackets provide the 95% confidence intervals.

Table 2.2. Number of Brook Trout caught, catch-per-minute, Brook Trout seconds, MaxN and Brook Trout DNA for each site and sampling year.

Site	Date	Brook Trout captured per 50m	Catch-per-minute	Brook Trout Seconds	MaxN	DNA (Copies per 5µL)
Dsouth	30-Jul-19	3	0.082	0	0	0.27
Walk6	01-Aug-19	8	0.133	4153	5	5.84
EW_17	08-Aug-19	17	0.276	27	1	5.05
EW_20	14-Aug-19	32	0.742	1	1	6.74
Walk10	15-Aug-19	39	1.242	354	2	1.64
EW1K	20-Aug-19	83	5.288	6139	5	55.95
YoungIn_Above	23-Aug-19	0	0	0	0	0.02
YoungIn_Below	23-Aug-19	7	0.190	1964	1	2.33
Dsouth	04-Aug-20	2	0.105	0	0	6.41
Walk10	05-Aug-20	0	0	0	0	2.78
EW1K	06-Aug-20	49	3.285	1260	1	467.33
YoungIn_Above	07-Aug-20	0	0	0	0	1.15
YoungIn_Below	07-Aug-20	13	0.685	1084	4	18.56
EW_17	10-Aug-20	14	0.142	1332	1	18.17
Walk6	11-Aug-20	10	0.296	1112	2	71.83
EW_20	12-Aug-20	21	0.408	2	1	57.57
Wwalk_S1L	13-Aug-20	4	0.091	0	0	15.38
160719	14-Aug-20	26	0.315	0	0	29.54

Chapter 2 Figures

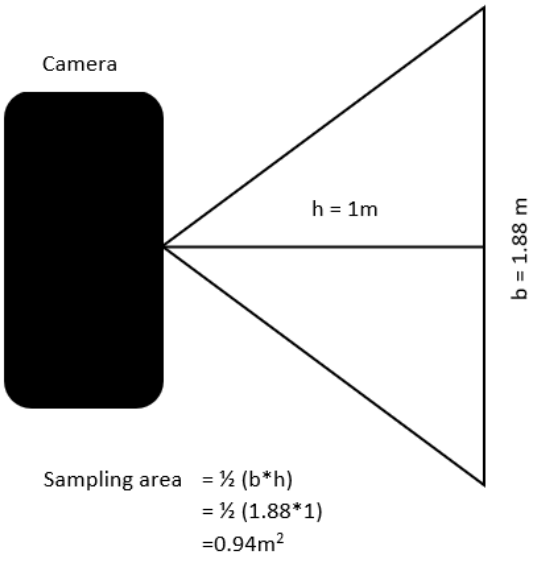

Figure 2.1. Visual representation of how the microhabitat sampling area was calculated (Leitrants 2020).

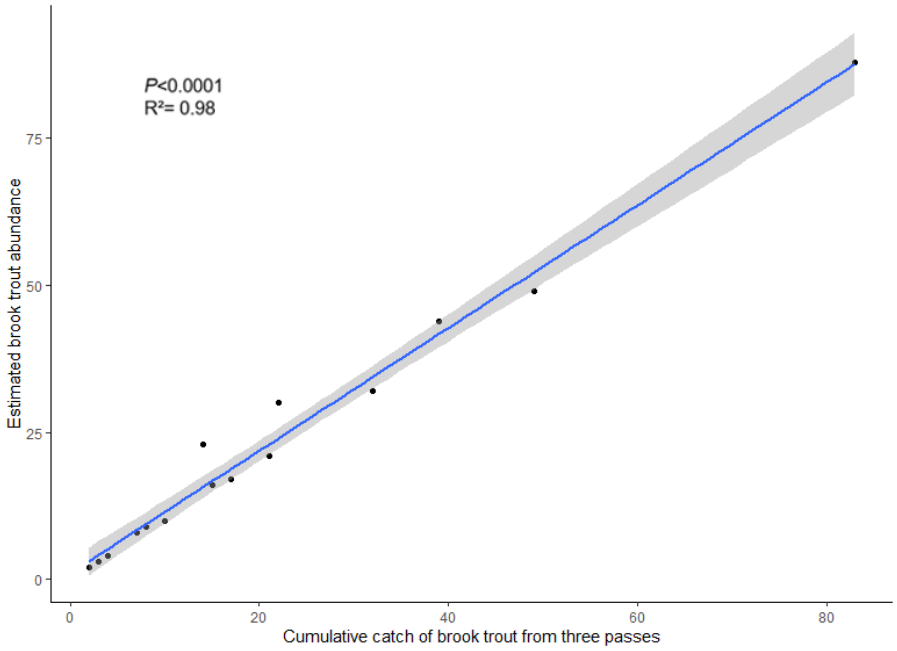

Figure 2.2. Relationship between the estimated number of Brook Trout abundance and the cumulative catch of Brook Trout from three passes of electrofishing ($F_{1,13}$=749, r^2=0.98 P<0.0001).

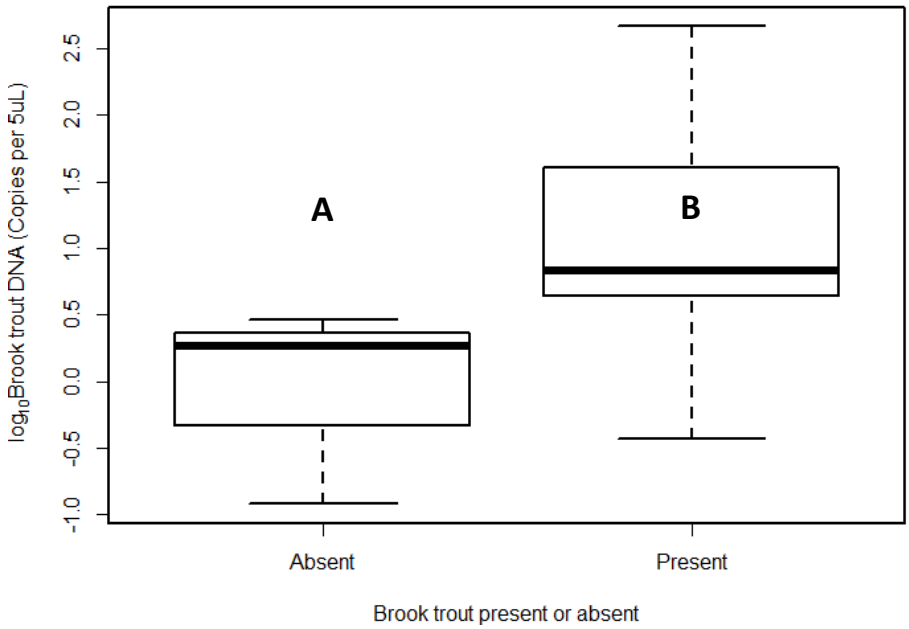

Figure 2.3. Environmental DNA copy numbers for streams with *Brook Trout* present or absent in electrofishing surveys. The letters indicate significant differences between groups. The mean number of DNA copies collected in *Brook Trout* absent streams was 1.32 copies per 5µL and the mean number of DNA copies collected in *Brook Trout* present streams was 50.05 copies per 5 µL µL (ANOVA $F_{1,16}=5.38$, $P=0.034$).

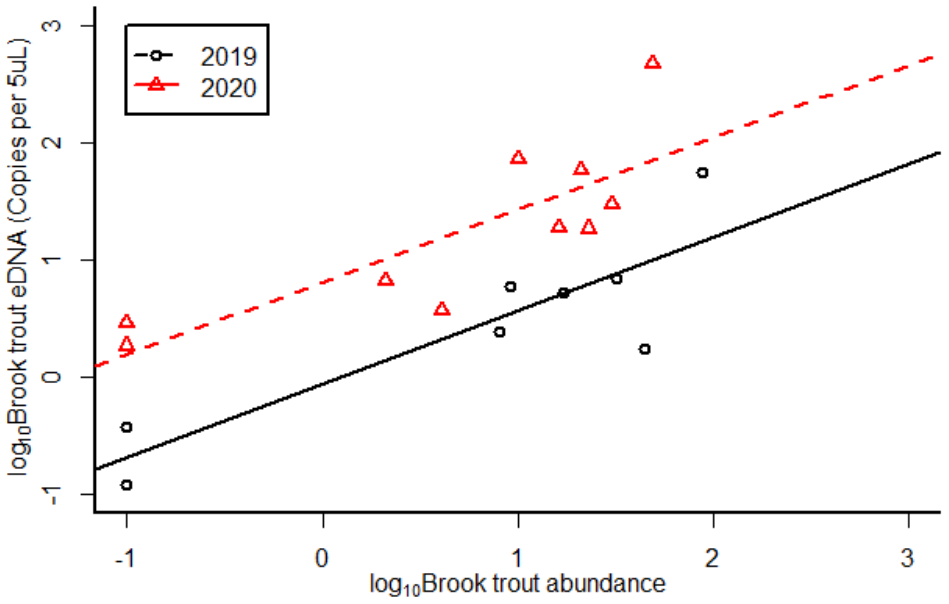

Figure 2.4. Estimated Brook Trout abundance-adjusted eDNA levels among sampling years. eDNA levels differed between sampling years ($F_{1,15}$= 27.2, P<0.001, r^2= 0.76) but the slope of the eDNA and estimated Brook Trout abundance relationship (slope = 0.62 eDNA copies per Brook Trout) did not differ between sampling years.

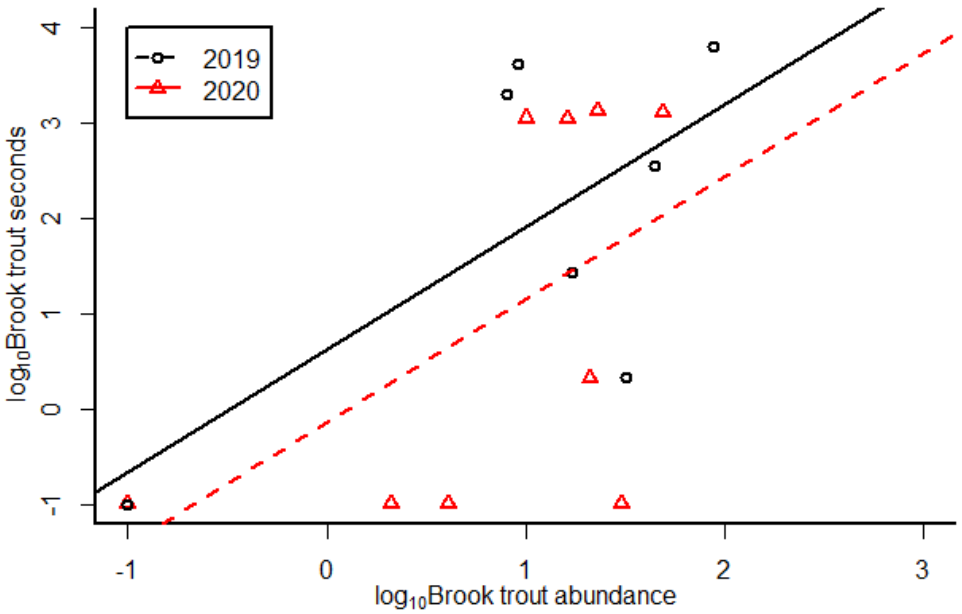

Figure 2.5. Estimated Brook Trout abundance-adjusted Brook Trout seconds among sampling years. Brook Trout seconds differed between sampling years ($F_{1,15}$= 7.05, P<0.01, r^2= 0.42) but the slope of the Brook Trout seconds, and estimated Brook Trout abundance relationship (slope = 1.29 seconds per Brook Trout) did not differ between sampling years.

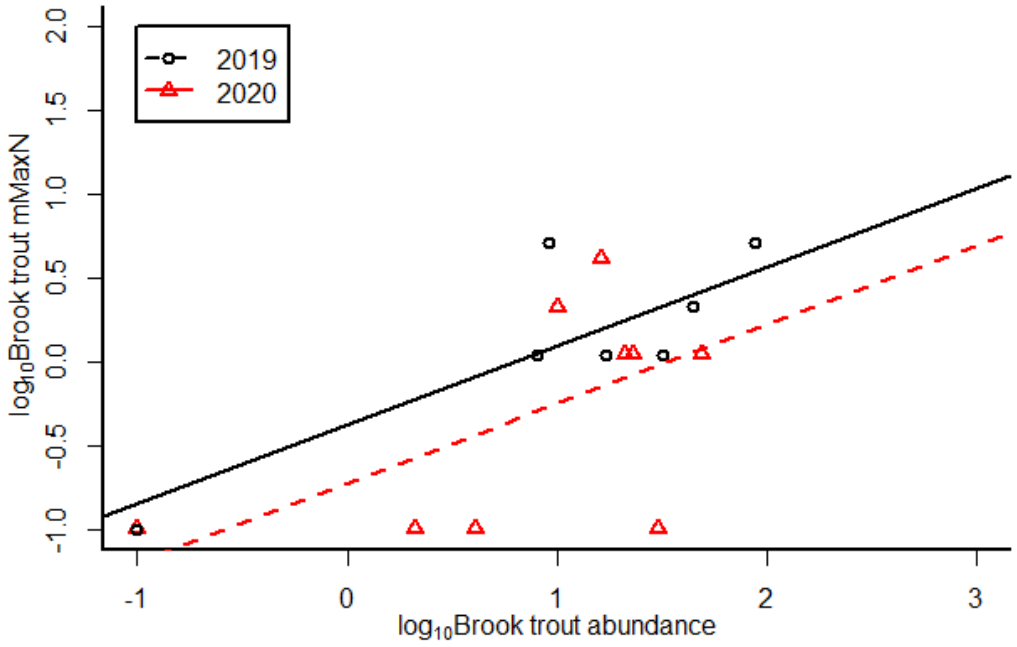

Figure 2.6. Estimated Brook Trout abundance-adjusted MaxN levels among sampling years. Brook Trout MaxN differed between sampling years ($F_{1,15}$= 11.24, P<0.01, r^2= 0.55) but the slope of the Brook Trout MaxN and estimated Brook Trout abundance relationship (slope = 0.47 MaxN counts per Brook Trout) did not differ between sampling years.

Chapter 3. Evaluating the factors influencing Brook Trout distribution at multiple spatial scales in Lake Superior tributaries

Abstract

Understanding habitat features that influence Brook Trout distributions at multiple spatial scales is inherently valuable for understanding complex habitat-fish relationships that may be overlooked if only assessing one spatial scale. The objectives of this study were (1) to determine which habitat features are associated with Brook Trout abundance measured at three spatial scales (microhabitat, reach and segment) and (2) to identify associations between specific stream habitat features and Brook Trout abundance that are unique to specific spatial scales or common among multiple spatial scales. Brook Trout abundance was quantified at the segment scale using eDNA concentrations in forty stream segments, using depletion models at eighteen reaches and underwater video cameras in sixty-eight microhabitat locations. Linear models selected with Akaike Information Criterion (AIC) determined that Brook Trout were associated with both scale-dependent habitat variables (canopy cover at the reach scale; baseflow index at the segment scale) and common habitat characteristics that were measured once (surface temperature, watershed size, stream discharge and width). Overall, this multi-scale analysis provides insight into the different stream habitat features that may best predict Brook Trout abundance at different scales. This information may be useful for fisheries managers as it identifies the ecological drivers that need to be maintained for Brook Trout to persist in streams in northwestern Ontario.

Introduction

Fish distributions and abundance can be affected by a combination of habitat characteristics that operate at multiple spatial and temporal scales in stream ecosystems. Aquatic habitats inherently exist in a hierarchy of organization, ranging from broad to fine scales (Deschênes and Rodríguez, 2007; Figure 3.1). For instance, a watershed contains rivers and streams, which contain microhabitats (Figure 3.1). Broad-scale (e.g., regional) factors (e.g., geology, climate) are likely to also affect fine-scale processes (e.g., microhabitat temperature ranges) as broad scale factors (e.g., climate) can potentially constrain the influence of fine scale factors (e.g., water temperature), all of which influence fish distributions to some extent (Chu et al., 2005; McKenna and Johnson, 2011; Steen et al., 2006). However, general habitat characteristics (e.g., water temperature) can be associated with fish distribution and abundance regardless of spatial scale, whereas certain habitat characteristics (e.g., woody debris or groundwater potential) may be unique to a specific spatial scale. Therefore, certain habitat characteristics can be seen as spatial filters of increasing spatial complexity through which aquatic species must pass to potentially be present at fine or broad spatial scales (Kwon et al., 2012).

A multi-scale approach to habitat assessment has the advantage of evaluating different scales of distribution-habitat relationships corresponding to characteristics (and their underlying biological processes) measured at appropriate spatial scales (Poizat and Pont, 1996; Hale et al., 2019). In aquatic environments, studies have examined the relationships between biotic and abiotic habitat factors, including geology, land cover, land use types, hydrology, and water quality as they relate to species distributions and abundances (McKenna and Johnson, 2011; Kwon et al., 2012; Kanno et al., 2015; Hale et

al., 2019). However, as a matter of convenience or survey methodology, typical fisheries research measures fish-habitat relationships at a single spatial scale, which may overlook species-habitat relationships at other (e.g., broader or finer) scales. Measuring both general habitat characteristics (e.g., water temperature) and unique habitat characteristics scaling from fine (e.g., woody debris) to broad scales (e.g., geology) and comparing those measurements with species distribution and abundance metrics can provide insight into the association of various habitat variables with species distributions and potential differences among scales.

Studying fish-habitat relationships across multiple spatial scales may provide a more complete picture of how species utilize various habitat characteristics and respond to changing habitat conditions (Deschênes and Rodríguez, 2007; Hale et al., 2019). Fisheries managers may use this information as a guide for assessing fish distribution and abundance and determining the appropriate spatial scale at which management decisions should be applied. For instance, if a general habitat characteristic (e.g., water temperature) is strongly associated with species abundance regardless of scale, managers may implement a uniform management approach to protect or enhance that characteristic across a broad region (Takashina and Baskett, 2015). Alternatively, if a unique habitat characteristic is strongly associated with a species at a specific spatial scale, then managers may consider using an approach tailored to that scale. Thus, identifying key habitat characteristics at a scale most relevant to the target species of interest is important for determining appropriate management strategies.

In streams, typical fisheries research is conducted within a reach sampling unit (50-100m in length; Figure 3.1C) as surveys at this scale can describe long-term effects of

human activities and determine population and distribution of aquatic communities using existing survey techniques (Fitzpatrick et al. 1998). Reaches contain specified features that are fairly uniform throughout, such as geomorphic characteristics (i.e., channel slope) and are bounded by specific locations on a stream such as changes in riparian or aquatic habitat, changes in channel or valley slope, and at junctions of major tributaries, to name a few (USDA, 2015). Changing seasonal abiotic (e.g., temperature; pH; turbidity; stream discharge) and biotic (e.g., intra/inter-species interactions) conditions can alter species behaviour and influence fish distributions at this reach scale (Raleigh, 1982; Kanno et al., 2014; Di Rocco et al., 2015). While information collected at a reach scale can answer many questions about fish populations, collecting information on fish populations at only the reach scale can result in insufficient data collection as only small sections of a watershed are surveyed and these sections may not be representative of the entire watershed.

By contrast, assessing a watershed drainage network system can provide insights on aquatic species distributions and abundances at a broader spatial scale than reaches (Figure 3.1A). A watershed may contain hundreds of tributaries, distinct regional biodiversity and broad patterns of aquatic ecosystem characteristics (Higgins et al., 2005). At this scale, fish distributions can be influenced by historical factors (e.g., postglacial dispersal), regional environmental factors (e.g., climate) and landscape features (e.g., waterfalls; Chu et al., 2005; Di Rocco et al., 2015; Hudy et al., 2008; McKenna & Johnson, 2011). Broad-scale environmental variables gradually change over long periods of time and are, thus, resistant to most acute, short-term anthropogenic factors (McKenna and Johnson, 2011). However, collecting information on fish-habitat relationships across an entire watershed can be inherently difficult due to the large geographic range of aquatic populations and limited

access for sampling. Thus, smaller subsections of a watershed drainage network known as stream segments (Figure 3.1B), may be used as a sampling unit to characterize ecosystems at the watershed level. Stream segments are more homogenous in physical, chemical and biological properties relative to its parent watershed network and are bounded by features such as stream confluence points where stream and water quality characteristics may change and landscape features such as waterfalls, landform features and significant changes in stream slope (Frissell et al., 1986). Stream segments incorporate multiple reaches, representing a wider range of habitat conditions within a stream.

The smallest scale generally evaluated within a reach is microhabitat (Figure 3.1D), distinctive patches with relatively homogenous substrate, water depth, and velocity (Frissell et al., 1986). Microhabitats are highly variable physical and abiotic environments, most evident in headwater tributaries, where seasonal fluctuations and inherent spatial heterogeneity present aquatic species with a range of rapidly changing microhabitat conditions (Poizat and Pont, 1996; Sotiropoulos et al., 2006a). Microhabitat analysis provides insight on localized habitat relationships, species interactions and the use of thermal refugia for stream fishes (Kanno et al., 2013). Although microhabitats are more prone to seasonal changes compared to broader spatial scales, they provide shelter and cover from predators, spawning grounds and critical thermal refuge zones during baseflow conditions (Poizat and Pont, 1996; Petty and Huntsman, 2012; Ecret and Mihuc, 2013).

Brook Trout (*Salvelinus fontinalis*) in Lake Superior tributaries is generally associated with streams that contain cold water temperatures from groundwater inputs, relatively stable water flows, and abundant stream cover (MacCrimmon and Campbell, 1969; Raleigh, 1982). While some habitat characteristics (e.g., water temperature) may

influence Brook Trout generally across spatial scales, other habitat characteristics are unique to a spatial scale (e.g., woody debris at the microhabitat scale, groundwater potential based on geology at the broad scale) and may provide additional information on the drivers of Brook Trout distribution and abundance. For example, at fine scales, Brook Trout is associated with cold microhabitat substrate temperatures, deep pooled areas with woody debris or overhanging stream banks for cover and thermal refugia (Raleigh, 1982; Neumann and Wildman, 2002; Sotiropoulos et al., 2006; Ecret and Mihuc, 2013). At the reach scale, Brook Trout is associated with cold water temperatures, abundant canopy cover, large streams, and stable stream discharges (Stranko et al., 2008; McKenna and Johnson, 2011; Alexiades and Fisher, 2015; DeWeber and Wagner, 2015). At broad scales, it may be most associated with land-cover types, stream slope and catchment area (McKenna and Johnson, 2011; Alexiades and Fisher, 2015; Di Rocco et al., 2015; Haxton et al., 2020). Thus, simultaneously assessing stream habitat features across multiple spatial scales as they relate to Brook Trout distribution and abundance can highlight important stream habitat- associations that may have been overlooked if just studying one spatial scale.

Brook Trout distribution cannot be examined at different spatial scales using the same data collection methods because data collected at fine scales may not provide the same information as broader scales (Schneider, 2001). Historically, electrofishing at the reach scale is the most common method of assessing stream Brook Trout. Electrofishing surveys provide abundance estimates of a reach, but not where fish are located within the reach, or if fish are distributed throughout the entire segment that the reach is located in. Further, the failure to capture Brook Trout in a reach does not necessarily confirm their

absence from a reach, as electrofishing surveys could have missed capturing individual Brook Trout or provide evidence that key microhabitat characteristics within a reach are present or absent. Using alternative sampling methods may provide insight on Brook Trout distribution at different habitat scales.

Species-specific DNA collected from raw environmental water samples has emerged recently as a tool for detecting the presence of aquatic organisms in stream environments (Baldigo et al., 2017; Lacoursière-Roussel et al., 2016). Environmental DNA (eDNA) techniques rely on the detection of genetic material originating from a target species that has been shed into the aquatic environment, rather than from detection based on direct contact with target individuals (Helbing & Hobbs, 2019; Lacoursière-Roussel et al., 2016; Rees et al., 2014). Elusive, cryptic or rare organisms can be reliably detected using eDNA and current studies are focused on further understanding eDNA dynamics in lentic, lotic and laboratory settings (Takahara et al., 2012; Thomsen et al., 2012; Jane et al. 2015; Wilcox et al., 2016). Environmental DNA may best represent broad-scale BBrook Trout distributions since eDNA varies in concentration within a stream and can potentially travel up to 200 m downstream from the target species (Jane et al., 2015). At a fine spatial scale, underwater video cameras (UWVC) have the power to visually detect Brook Trout occurrence in microhabitats and provide insight on where Brook Trout are located in a reach. Combining eDNA, UWVC and electrofishing sampling techniques can provide more information on Brook Trout presence, absence, distribution, and abundance in a stream system, and at various relevant scales, than any single method in isolation.

The objectives of this study were (1) to determine which habitat variables are most associated with estimates of Brook Trout abundance measured at three spatial scales

(microhabitat, reach and segment) and (2) to determine if these associations are scale dependent (i.e., unique to a spatial scale) or are similar among different spatial scales. This approach may provide insight into important habitat characteristics for Brook Trout and the most appropriate scale, or scales, to assess Brook Trout distribution and abundance.

Methods

Thirty stream segments were surveyed from 2019 to 2020 and were selected based on level of accessibility, and previous survey assessments conducted by CAEP at the CNFER (Appendix A-1; Table 3.1). Ten of the segments were sampled in both 2019 and 2020 for eDNA, and underwater video surveys, creating forty eDNA surveys in total and eighty underwater video surveys as two microhabitats per segment were surveyed in each segment (Table 3.1). Due to the restrictions of COVID-19 and the delay in starting field sampling in 2020, only a selected number of reaches were chosen for electrofishing which, were reasonably accessible and were previously surveyed in 2019 (8 reaches were electrofished in both 2019 and 2020 plus an additional 2 reaches in 2020; Table 3.1; Appendix A-1). Only sixty-eight microhabitat surveys were used for analysis due to technical errors with the Go Pro cameras. All surveys were treated as independent in the analyses with sampling year (2019, 2020) included as a factor.

1. Habitat Surveys

1.1 Segments

Segment-scale habitat surveys relied on previously collected information by CAEP at the CNFER. Watershed areas, upstream of the downstream end of each segment, were delineated for all eDNA sample sites using a 30-metre cell resolution Enhanced Flow

Direction Grid (OFAT, 2013). The OFAT generated the following watershed characteristics used as potential predictor variables in the models: slope (%), base flow index, and percentage of forest cover types for the watershed. Baseflow index is a measure of the ratio of long-term baseflow to total stream flow and represents the slow continuous contribution of groundwater to river flow (OFAT; MNRF Ontario Flow Assessment Tool, 2013; Bloomfield, 2009). Land-cover types (sparse treed land, treed upland, deciduous treed, mixed treed and coniferous treed land) were used to determine the overall percentage of forest cover for the watershed (OFAT, 2013). In total, thirty stream segments were surveyed (10 segments were surveyed twice) in this study and 3 unique broad-scale response variables (% slope, % forested land cover and baseflow index; Figure 3.2) were used in the broad-scale models (Table 3.2; Appendix A-3).

1.2 Reaches

Beginning at the most downstream point of the 50 m reach, 5 transects were placed at 10 m intervals upstream across the channel. Habitat covariates were measured at 5 equidistant points across each transect (Figure 3.3). At each equidistant point, depth (mm) was measured with a meter stick, and substrate temperature (°C) was measured with a Therma Plus Meter and Probe (ThermoWorks, American Fork, Utah). Riparian canopy cover (%) was measured with a densiometer at the midpoint of the first, middle and last transect and averaged across the reach while substrate temperature and depth were. averaged across all the transects within the reach. In total, ten unique reaches were surveyed (18 reach surveys were conducted in total, 8 surveys were conducted in 2019 and 2020 at the same reach and 2 additional reaches were surveyed in 2020) and 3 reach-scale

response variables (mean substrate temperature, mean depth and mean riparian canopy cover) were used in the reach- scale models (Table 3.2; Appendix A-2).

1.3 Microhabitats

Habitat surveys were conducted at 2 microhabitat locations within the 50m reach. Two Go Pro Hero 5 cameras (GoPro, San Mateo, California) mounted on Manfrotto Compact Light tripods (Manfrotto, Markham, Ontario) were placed facing upstream in two chosen locations that shared similar habitat characteristics (i.e. substrate type, woody debris, depth). A metal stake with flagging tape was placed 1 m from the camera lens to delineate the microhabitat sample area which was the camera's field of view up to the stake, an area of 0.94m2 (Leitrants 2020; Figure 2.1). Cameras were placed in areas free of obstruction from large boulders, downed woody debris and vegetation. Each camera started recording at the same time and recorded for 1-hour intervals in 1080p linear view after a 15-minute settling period. In total, sixty-eight microhabitat locations were used in this survey as two microhabitats were located within each of the forty segments. The habitat variables recorded at the microhabitat-scale included: substrate temperature, depth, and canopy cover. Substrate temperature was measured in nine locations within the microhabitat (three measurements were taken to the left of the camera, at the camera and to the right of the camera; three measurements were taken at the midpoint between the camera and the flag at the left, middle and right; and measurements were taken to the left of the flag, at the flag and to the right of the flag (Figure 3.4). Depth was measured in three locations within the microhabitat (at the camera, at the midpoint between the camera and the flag and at the flag, Figure 3.4). Substrate temperature and depth measurements were averaged across the microhabitat. Riparian canopy cover was measured at one location within the microhabitat

at the camera location. Three microhabitat-scale habitat variables (substrate temperature, depth, and canopy cover) were used in the models (Table 3.2; Appendix A-4). Underwater video surveys were conducted from July 2 – August 26, 2019, and July 15 – September 2, 2020.

1.4 Common Habitat Variables

Four habitat variables were indicative of habitat conditions across the stream segment and do not fluctuate based on the spatial scale. These common habitat variables were included in each model: stream width (m), stream discharge (m^3/s), surface temperature (°C) and watershed size (km^2; Table 3.2). Stream width (m) was averaged over three measurements taken along the first, middle and fifth transect of each reach. Stream discharge was measured at each transect by dividing the stream width into 10 (or 20) equal intervals. At each interval, depth was measured, and velocity was taken at 60% of the measured depth. To calculate discharge of each interval, the interval cross-sectional area was calculated (interval width x depth) and multiplied by the interval velocity. The discharge values of all intervals were summed to calculate total stream discharge. Flow velocity (m/s) was measured with a Marsh 2000 Flo-Mate handheld electromagnetic water flow meter (Hach 2020; Table 3.2). Surface temperature was measured at the midpoint of the first transect. Watershed size, delineated from the eDNA sampling location at the downstream point of each segment, was common across scales as it does not change regardless of the spatial scale being surveyed.

2. Brook Trout abundance measurements

2.1 Segments: Environmental DNA sampling

Environmental DNA samples were taken immediately upon arrival at the site, before any crew members or equipment entered the stream, to reduce the chance of contamination. Within the same 50 m reach that electrofishing and UWVC surveys were subsequently conducted, triplicate 1-L water samples were collected using sterilized Nalgene bottles at three equidistant points along the farthest downstream transect of the reach. Prior to sample collection, Nalgene bottles were placed in 10% bleach solution for 30 minutes, rinsed with double-deionized water and left to dry. Once dried, bottles were paired with their lids and sealed. A Nalgene bottle was filled with only distilled water, labelled as the 'blank' sample and was carried within the cooler to the site to assure that no contamination occurred from the lab. Once at the site, Nalgene bottles were triple rinsed with stream water to wash away residual double-deionized water from the lab. Bottles were pre-labelled with the site name, sample number and time of collection. The field samples were placed inside the cooler with sterilized ice packs and transported back to the lab for filtration.

Water samples were filtered following the protocols provided by Wilson et al. (2014). Pre- and post-filtration control filters were used for every sample to confirm that filter equipment was not a source of eDNA. While wearing powderless nitrile disposable gloves, filters funnels were placed in 10% bleach solution for 20 minutes between samples and rinsed thoroughly with double deionized water. Two forceps per sample were sterilized with 70% ethanol and lit on fire to burn any residual ethanol to extinction. The sterilized forceps were then used to place Whatman GF/C 1.2 μm pore size filter membranes on the funnel base. Water samples were poured into the funnel (one water sample per funnel) and the EZ-Stream pump (Millipore EZ-Stream) was turned on to allow the water to filter

through. Once the 1-L water sample was filtered, a new pair of sterilized forceps was used to roll up and transfer the filter membrane to a labelled, pre-sterilized 15mL sample storage container that contained 1mL of Longmire solution (a lysis buffer that is used for the preservation of eDNA). Lastly, the 'blank' distilled water sample was filtered to make sure that no contamination occurred during transfer. Environmental DNA extraction was conducted using the MoBio PowerWater DNA Isolation kit (MobBio Laboratories, Inc) at Trent University. The BRK2 primer was used for genetic testing of Brook Trout DNA using qPCR (Wilson et al., 2014). Brook Trout environmental DNA levels were recorded as the number of DNA copies per 5 µL. Any DNA level that was less than 0.07 copy per 5 µL was considered a non-detection (Wilson et al. 2014a).

2.2 Reaches: Electrofishing

After taking eDNA samples, but prior to UWVC surveys and electrofishing surveys, 15 m block nets were placed perpendicular to the direction of stream flow at the upstream and downstream ends of a 50 m reach to prevent fish from escaping the survey area. Following UWVC surveys and eDNA sampling, triple pass electrofishing was conducted within the same blocked off 50 m reach, using a battery powered backpack electrofisher with pulsed direct current (Model LR-24; Smith-Root, Vancouver, Washington). While starting at the downstream block net and moving upstream, horizontal sweeps across the stream width were performed until the entire reach had been covered with 2 netters on either side of the operator. All captured fish from each pass were placed in aerated buckets containing ambient stream temperature water. Two more electrofishing passes were conducted using the same methods. The site name, date, and water conductivity were recorded, and the start time and shocking seconds were recorded after

each of the three passes. Brook Trout from each pass were counted, measured for total length and fork length, and weighed. All other species caught were identified to species, counted, and weighed in bulk. All fish were released back into the stream once the block nets were removed. Electrofishing surveys were used to determine Brook Trout abundance estimates from depletion models (see below).

3. Data Analysis

To estimate Brook Trout abundance, a depletion method known as the "k-pass" removal method was used (Carle and Strub 1978). After each electrofishing "pass", the number of Brook Trout were recorded and were physically removed from the population. Thus, under certain assumptions (i.e., the population of Brook Trout is closed, and the probability of Brook Trout capture (*p)* is constant for all animals and from sample to sample) the overall population size and probability of capture was estimated from the number of Brook Trout successively removed. These assumptions were met in the current study by closing off the surveyed stream section with block nets, and by maintaining consistent sampling effort across electrofishing passes. All analyses were conducted using the statistical software package R (Version 4.2.0; R Core Team 2022) The R package "FSA: Simple Fisheries Stock Assessment Methods" (Ogle et al. 2022) and the *removal()* function that defaults to the Carle and Strub method was used to estimate Brook Trout abundance and capture probability (Carle and Strub 1978).

To study relationships between Brook Trout and habitat characteristics measured across multiple spatial scales, Akaike Information Criterion (AIC) was used to determine the best model among a set of models with different combinations of habitat variables. The statistical software R (Version 4.2.0) was used. Relationships between habitat

characteristics and Brook Trout abundance measurements were analyzed separately for each spatial scale (Akaike, 1973). The dependent variables (estimated Brook Trout abundance, BT seconds and Brook Trout eDNA) were $\log_{10}$ transformed to meet the assumptions of data normality after visually inspecting the spread of the data through histogram plots and diagnostic plots. No transformations were required for the habitat variables as they met the assumptions of linearity, confirmed by diagnostic plots. Residual plots were generated to ensure normal distribution of the data and homogeneity of residuals for the models. Additionally, to ensure variables were not highly correlated with one another, scatterplots were generated to check for multicollinearity. Variance inflation factors, a measure of how much variance of an independent variable is influenced or inflated by another variable that is correlated with the other independent variables, were checked by refitting models to identify the next highest ranked variables until all variables reached a variance inflation factor <2 (Zuur et al. 2010). Residual plots were generated to ensure normal distribution of the data and homogeneity of residuals for all the top models.

In the microhabitat models, substrate temperature was excluded due to a high VIF and, in the reach-scale models, stream discharge, stream width and surface water temperature were excluded from the models due to a high VIF. After those variables were excluded, models of all combinations of stream habitat variables were generated, the top models were determined by the lowest AICc value and adjusted R^2 values (Akaike, 1973). AICc values were calculated from AIC scores corrected for small sample sizes for the top 10 models and delta AICc values were generated to see the differences in AICc scores between models (Akaike, 1973). Additionally, AICc weights were determined for top 10 models (Akaike, 1973).

Results

At the segment scale, Brook Trout eDNA ranged from 0 to 553.84 copies per 5µL (mean Brook Trout eDNA= 58.11 ± 125.19 copies per 5µL; see Table 3.3 for segment-scale habitat measurements). The best fit model at the segment scale carrying 32.7% of the AICc model weights, included surface temperature, BFI and watershed size (AICc = 103.99, r^2= 0.187; Table 3.4; Figure 3.2). However, the next top AICc model carried 20% of the AICc model weights and was less than 1 AICc unit different than the top model and included surface temperature, and BFI (AICc=104.97, r^2 = 0.133; Table 3.4). The next model included surface temperature and stream width but dropped considerably in AICc model weights with a weight of 9% but was still less than 5 AICc units different from the top model (AICc=106.57, r^2 = 0.099; Table 3.4). The next models gradually drop in AICc model weights and increase in Delta AICc values (Table 3.4). In 7 of the 10 top models, surface water temperature was included and baseflow index was included in the top 2 models suggesting that these common habitat variables (specifically surface water temperature, and baseflow index) best explain the heterogeneity in Brook Trout eDNA concentrations.

At the ten sites surveyed at the reach scale, Brook Trout abundance estimates ranged from 0 to 86 Brook Trout (see Table 3.5 for reach-scale habitat measurements). Most of the common habitat variables were dropped on the basis of variance inflation factors (i.e., stream discharge, stream width and surface water temperature). The best-fit model carried 28% of the AICc model weights included the unique reach-scale variable mean riparian canopy cover (AICc = 52.93, r^2=0.068; Table 3.6, Figure 3.3). The second-best model included the unique reach-scale variable canopy cover and the common habitat variable watershed size and carried 20% of the AICc model weights and explained more of

the variability in the dependent variable (AICc = 53.63, r^2=0.143; Table 3.6). Six of the 10 top models included canopy cover which suggests it is an important variable in describing the heterogeneity in estimated Brook Trout abundance (Table 3.6). The third-best model carried 12% of the AICc model weights and included the common variable watershed size but did not explain any of the variability in the dependent variable (AICc=54.56, r^2=-0.02; Table 3.6). The remaining models drop considerably in AICc model weights and coefficient of determination values which suggest that canopy cover and watershed size are strongly associated with Brook Trout abundance at the reach scale.

At the 68 sites surveyed at the microhabitat scale, Brook Trout seconds ranged from 0 to 3948 seconds (see Table 3.7 for microhabitat-scale habitat measurements). The best-fit model, carrying 39% of the AICc model weights included the common variables surface temperature and discharge (AICc=252.77, r^2=0.134; Table 3.8; Figure 3.4). The second-best model carried 23% of the AICc model weights and included the common variables: surface temperature and width (AICc=253.84, r^2=0.12; Table 3.8). Finally, the third-best model included the common variables surface temperature, discharge and width and carried 12% of the AICc model weights (AICc = 255.11, r^2=0.12; Table 3.8). After the third-best model, there was a considerable drop in AICc model weights and increased delta AIC values. Out of the top 10 models, 7 of them included surface temperature, suggesting that surface temperature is an important variable explaining the heterogeneity in Brook Trout abundance. Further, in all 10 models, a common habitat variable was included (surface water temperature, discharge and stream width) suggesting that these common habitat variables are important in describing Brook Trout abundance at the microhabitat scale.

Discussion

Brook Trout in stream systems are associated with several habitat characteristics (e.g., the availability of canopy cover, groundwater inputs, stable discharges that provide highly oxygenated water, pool and riffle habitats that provide cold water temperatures and cover) that influence abundance and distribution at different spatial scales (Raleigh, 1982; Wehrly et al., 2007; Waco and Taylor, 2010; Petty et al., 2012; Ecret and Mihuc, 2013). My multi-scale analysis determined that surface water temperature, baseflow indices and stream width were most associated with Brook Trout at the segment scale, canopy cover and watershed size was most associated with Brook Trout at the reach scale, and surface temperature, stream discharge and stream width were strongly associated with Brook Trout at the microhabitat scale. These results indicate that Brook Trout habitat associations at all spatial scales can include both scale-specific variables and general habitat characteristics.

At the segment scale, both a unique watershed-level habitat variable (baseflow index) and common habitat variables (surface water temperature and stream width) emerge as important habitat variables for Brook Trout. Similarly, at the microhabitat scale, common habitat variables (surface temperature, stream width and stream discharge) emerge as important habitat variables for Brook Trout. At both the segment and microhabitat scale, Brook Trout occupancy time and eDNA levels were greater when surface water temperatures were colder. This finding is not surprising as one of the most important habitat characteristics is the availability of an abundant supply of clean, cold, well-oxygenated water (Raleigh, 1982). Cold water temperatures are critically important for Brook Trout in stream environments, especially during baseflow conditions where warmer stream temperatures pose a significant threat to Brook Trout survival (Sotiropoulos et al., 2006b; Deschênes and Rodríguez, 2007; Xu et al., 2010; Petty and Huntsman, 2012).

In some cases, Brook Trout can tolerate stream temperatures up to 24°C but mostly prefer a temperature range of 10-16°C as growth and survival are expected at these temperatures (MacCrimmon and Campbell, 1969; Raleigh, 1982; Baird and Krueger, 2003; Stranko et al., 2008). The highest surface temperature that I recorded was 21.3°C and, on average surface temperatures were 16.3°C, a preferred temperature for Brook Trout (Raleigh, 1982). Overall, stream temperature is an important management and conservation consideration for Brook Trout regardless of the spatial scale of management decisions.

Baseflow index emerged as an important habitat characteristic describing a positive relationship with Brook Trout abundance at the segment scale. Higher baseflow indices are correlated with lower stream temperatures and linked to the amount of groundwater flow entering a stream system (Chang and Psaris, 2013). Brook Trout distribution within a watershed tend to be linked to groundwater inputs which aid in maintaining consistently cool water conditions in the summer, warm water conditions in the winter to protect spawning areas from freezing and overall stable thermal conditions, as such, one of the best indicators of Brook Trout at a watershed scale is geological characteristics related to groundwater (Kanno et al., 2014; Stanfield et al., 2006; Chu et al., 2008). Most of the stream segments surveyed had a baseflow index greater than 50% and according to past studies, a base flow of 55% of the average annual daily flow has been reported to be optimal for Brook Trout (Binns and Eiserman, 1979; Wesche, 1980). Further, groundwater discharge areas are a major requirement for selecting spawning habitat by Brook Trout (Curry and Noakes, 1995). Landcover type and land use influence groundwater levels by potentially reducing groundwater recharge areas which could impact the supply of water to the stream system, thus reducing stream baseflow (Waco & Taylor, 2010). Watersheds

with high groundwater discharge may be less sensitive to the potential effects of climate change. For Brook Trout, groundwater flow was found to be a limiting factor for populations in a study in Pigeon River, Michigan highlighting the importance of baseflow indices for describing the distribution of Brook Trout populations (Chu et al., 2008; Benson, 1953).

Brook Trout abundance, measured by triple-pass electrofishing, was strongly associated with riparian canopy cover measured at the reach scale and watershed size, a common habitat variable. No associations between canopy cover measured at the microhabitat scale and Brook Trout abundance suggesting that riparian canopy cover is more influential to Brook Trout at the broader reach scale. Although canopy cover varied among sites, more Brook Trout were captured at sites with greater canopy cover. This pattern is consistent with several studies that determined that canopy cover is an essential component of Brook Trout habitat and is correlated with lower stream temperatures, a critical habitat requirement for Brook Trout (Petty et al., 2005; Deschênes and Rodríguez, 2007; McKenna and Johnson, 2011; Dugdale et al., 2018). Although substrate temperature did not emerge as an important variable at the reach scale, it may have been masked as temporally variable and may only represents habitat conditions at a small point in time. Additionally, if all temperature measurements are below the critical thresholds for Brook Trout, then temperature may not be informative at the time of the survey. Thus, the influence of stream characteristics like water temperature, may not have been detected and may reduce the ability of detecting unique contributions of temperature, whereas other habitat features that were more variable across microhabitats may provide more information on Brook Trout-microhabitat associations. In Hart Brook, a tributary of Lake

Ontario, water depth and canopy cover were the major habitat variables influencing use by Brook Trout great than one year of age (Johnson et al., 2016). Additionally, Brook Trout in Algonquin Park were consistently observed in areas of moderate riparian canopy cover (57-86%; Biro, 2008) whereas in this study, Brook Trout were captured in a variety of riparian canopy cover measurements (1-99%) but were captured in higher numbers in streams with >16% coverage.

The relative importance of canopy cover has been observed in several studies documenting Brook Trout population declines in areas where landscape-level forest cover changes have occurred (Hudy et al., 2008; Stranko et al., 2008; McKenna and Johnson, 2011; DeWeber and Wagner, 2015). Although no associations between Brook Trout and forest land cover at the segment scale was found, the repercussions of changes in landscape-level forest cover (e.g., due to forest harvest adjacent to streams) may reduce canopy cover and impact Brook Trout populations at finer spatial scales. For example, reductions in forest cover caused by agricultural activities, logging, and landscape development have caused increases in impervious land cover, higher water temperatures, increased sediment loads, and higher rates of erosion, all factors that have contributed to declines in Brook Trout populations (MacCrimmon and Campbell, 1969; Hudy et al., 2008; Stranko et al., 2008; McKenna and Johnson, 2011). The loss of forested land cover is one of the main drivers influencing the degradation of stream quality generally (Booth et al., 2002). Therefore, to maintain and improve Brook Trout populations, the results of this study suggest that fisheries managers should protect riparian canopy cover and monitor landscape-level changes if groundwater potential is limiting.

Brook Trout rely on clean, cold, well-oxygenated water and are highly sensitive to the effects of broad-scale stressors including climate change, introduced species and, watershed development (Hudy et al., 2008, McKenna and Johnson, 2011, Chu et al., 2005). Because Brook Trout thrive in cold, clear stream conditions (MacCrimmon and Campbell, 1969; Raleigh, 1982), the higher eDNA levels in colder stream water is not surprising. As water temperatures affect all life history stages of Brook Trout and their activity level during different seasons, eDNA levels should be interpreted based on temperature. For example, a strong correlation was observed between water temperature and common carp eDNA levels, with higher concentrations found in warmer water conditions (Takahara et al., 2012). Thus, higher eDNA levels could be due to the overall life history and habitat preference of the target organism and when interpreting Brook Trout eDNA, species biology and habitat conditions need to be considered to account for differences in eDNA shedding and degradation. As eDNA samples were taken when surface water temperatures were suitable for Brook Trout persistence, eDNA levels may be higher as colder water temperatures enable Brook Trout movement and active fish tend to shed more DNA (Petty et al., 2012; Thalinger et al., 2021). Future eDNA samples should be taken in spring and fall to better understand the activity levels of Brook Trout and their differences in DNA shedding based on seasonal fluctuations.

Watershed size was an important variable describing Brook Trout abundance at the segment and reach scales. Brook Trout individuals and eDNA concentrations were found in watersheds that ranged from 1km^2 to 59km^2. However, high variability in both estimated Brook Trout abundance and eDNA concentrations were found among watershed sizes, but the highest amount of eDNA and number of Brook Trout were found in smaller

watersheds. Smaller watersheds have less surface runoff potential which reduces the amount of sediment and nutrient loading in streams compared to larger watersheds (Holdren et al., 2001). Thus, higher Brook Trout eDNA concentrations and abundance estimates in smaller watersheds may have been due to the favourable conditions that Brook Trout prefer (i.e., cold, clear streams) provided by smaller watersheds. Therefore, stream management practices cannot ignore the influence of watershed size since they are inherently linked to suitable water conditions for Brook Trout. As anthropogenic activities can occur in any watershed, large or small, and the influence of each activity can vary from watershed to watershed, managing stream systems for sensitive species like Brook Trout should start with understanding the attributes of the watershed and the types of use and activities occurring on the landscape (Holdren et al., 2001).

A strong association between Brook Trout abundance and stream discharge was only observed at the at the microhabitat scale. Brook Trout abundance was higher in areas with lower stream discharges. Previous studies have shown that stable water flows and relatively slow stream velocity are an important requirement for Brook Trout in riverine habitats (Raleigh, 1982; Johnson et al., 2016). However, changes in flow regimes and water availability could reduce Brook Trout population numbers or alter distributions, causing them to seek areas with ample water supply, food availability and stable water flows (Adams et al., 2008). Brook Trout seconds and MaxN in microhabitats may have been higher in lower discharge streams if water volume was limited in the rest of the reach. This may have caused Brook Trout to be “clumped” in pools that are relatively deeper and colder than other areas in the reach. In the reaches surveyed, at least two pool habitats were available in every 50 m reach as the underwater cameras were placed in these

habitats. It is important to monitor stream discharge in conjunction with stream water temperatures as they can quickly match ambient air temperatures, causing cold-water streams to warm at a higher rate, which could negatively impact Brook Trout survival (Nuhfer et al., 2017). For future Brook Trout populations to thrive, stable stream flows that provide cold, clear water conditions must be available.

At the segment and microhabitat scale, higher levels of Brook Trout eDNA and abundance in microhabitats were found in streams less than 3m in width. In theory, when streams are narrower and flow velocity is lower, greater eDNA levels will be collected as the rate of dilution is reduced (Curtis et al., 2020). Therefore, it is not surprising that higher eDNA levels were found in narrower streams with slower stream flows. Further, Brook Trout prefer streams with ample groundwater and cold-water temperatures (Baird and Krueger, 2003; Waco and Taylor, 2010). Headwater streams generally have relatively higher groundwater input than second or third order streams, therefore Brook Trout may prefer to occupy these areas, especially during baseflow conditions (Kanno et al., 2015). Understanding how abiotic conditions influence the biology of the target organism is crucial for understanding eDNA results. For example, Jane et al. (2015) introduced caged Brook Trout into two fishless headwater streams and found detectable levels of Brook Trout ~240m downstream of the cages when flows were relatively low. As such, eDNA techniques are debated as a reliable method for determining organism abundance in small headwater streams as it is challenging to pinpoint the origin of eDNA along with the habitat conditions that influence degradation (Jane et al., 2015). Coupling eDNA information with the underwater video surveys could provide information on whether

Brook Trout are occupying these narrower streams and how they are behaving which may be reflected in eDNA levels.

Overall, the multi-scale approach helped identify the relative importance of stream habitat characteristics measured at three spatial scales on the distribution and abundance of Brook Trout. I found that certain habitat characteristics are more important for Brook Trout regardless of scale but there are characteristics that help explain Brook Trout abundance that are unique to some scales. However, at both the reach and segment scale, combinations of unique and common habitat variables were found to be associated with Brook Trout abundance. Surface water temperature and stream width were found to be important factors associating with Brook Trout at broad and fine spatial scales while other habitat features, like canopy cover and baseflow index, had a stronger association with Brook Trout at specific spatial scales. Fisheries management, may value the evidence of Brook Trout- habitat relationships at all spatial scales to fill in the gaps of areas that are under surveyed. Overall, this multi-scale analysis identified different stream habitat features that may best predict Brook Trout abundance at different scales and may be utilized by fisheries managers to decide which habitat factors should be managed. Future studies should explore the influence of landscape level disturbance (i.e., forestry and/or urbanization) within a watershed as predictor variables for Brook Trout at different spatial scales to further understand the influence of broad-scale impacts on Brook Trout populations.

Chapter 3 Tables

Table 3.1. Table showing the sites used in this survey and the survey type conducted in 2019 and 2020. Thirty streams were surveyed in this study, but ten streams were sampled in both 2019 and 2020 for eDNA and underwater videos. Eight streams were electrofished in 2019 and the same eight were surveyed in 2020 with an additional two streams.

Site Name	eDNA	Electrofishing	Underwater video
Walk10	2019, 2020	2019, 2020	2019, 2020
EW_20	2019, 2020	2019, 2020	2019, 2020
Wwalk_S1L	2019, 2020	2020	2019, 2020
EW_17	2019, 2020	2019, 2020	2019, 2020
160719	2019, 2020	2020	2019, 2020
Dsouth	2019, 2020	2019, 2020	2019, 2020
Walk6	2019, 2020	2019, 2020	2019, 2020
EW1K	2019, 2020	2019, 2020	2019, 2020
YoungIn_Below	2019, 2020	2019, 2020	2019, 2020
YoungIn_Above	2019, 2020	2019, 2020	2019, 2020
EW_3	2020	N/A	2020
SwardBigP_50k_L	2020	N/A	2020
SwardBigP_50k_S	2020	N/A	2020
Pine_10k	2020	N/A	2020
DnorBridge	2020	N/A	2020
Nicholson_50k_L	2020	N/A	2020
150719	2019	N/A	2019
Fur_1S	2020	N/A	2020
Fur_1L	2020	N/A	2020
SamAs_20k_L	2020	N/A	2020
Walk4.2	2019	N/A	2019
Seagull_10k_L	2020	N/A	2020
DriftstoneTrib_7k_L	2020	N/A	2020
DriftstoneTrib_7k_S	2020	N/A	2020
Beaverhide_14k	2020	N/A	2020
Dam_1k	2019	N/A	2019
Ouimet_10_2	2020	N/A	2020
170719	2019	N/A	2019
EscQui_2Wf	2020	N/A	2020
EscQui_Bx	2020	N/A	2020

Table 3.2. Unique and common stream habitat variables measured at the segment, reach and microhabitat scales. Forested land cover (%), slope (%), baseflow index and watershed size were generated using the Ontario Flow Assessment Tool (OFAT, 2013). The other habitat variables were measured *in situ.*

Spatial Scale	**Unique Spatial Scale Variables**	**Common Variables Measured Across Spatial Scales**
Segment (>50m)	▪ Forested Land Cover (%) ▪ Slope (%) ▪ Baseflow index (BFI)	▪ Stream Discharge (m^3/s) ▪ Surface Temperature (°C) ▪ Stream Width (m) ▪ Watershed size (km^2)
Reach (50m)	▪ Mean Canopy Cover (%) ▪ Mean Substrate Temperature (°C) ▪ Mean Depth (mm)	
Microhabitat ($1m^2$)	▪ Canopy Cover (%) ▪ Substrate Temperature (°C) ▪ Depth (mm) ▪ Camera location (cold or warm)	

Table 3.3. Segment-scale habitat measurements. Stream slope, forested land cover and baseflow index were used as scale-specific independent variables in the segment-scale models.

Segment-scale habitat variables	Mean	Min- Max	Standard Error
Slope (%)	1.1	0 – 2.34	± 0.95
Forested land cover (%)	75.3	30.6 – 99.5	± 17.2
Baseflow Index	0.53	0.50 – 0.71	± 0.001
Surface temperature (°C)	15.3	7.1 – 20.3	± 3.0
Stream width (m)	3.1	0.45 – 6.83	± 1.86
Stream discharge (m^3/s)	0.033	0.009 – 0.10	± 0.033

Table 3.4. Top weighted AICc models explaining the heterogeneity in segment-scale Brook Trout eDNA.

Model Variables	AICc	Delta AICc	AICc weight	Adjusted R^2
Surface Temperature + Watershed size + Baseflow Index	103.99	0.0000	0.33	0.187
Surface temperature + Baseflow Index	104.97	0.97	0.20	0.133
Surface temperature + Width	106.57	2.57	0.090	0.099
Surface temperature + Watershed size	106.71	2.71	0.084	0.133
Width + Slope + Watershed size	106.78	2.78	0.081	0.131
Width + Landcover + Watershed size + Baseflow Index	106.82	2.83	0.080	0.026
Surface temperature + Width + Slope	107.72	3.73	0.051	0.11
Surface temperature + Discharge + Watershed size + Slope	108.85	4.85	0.029	0.17
Discharge + Surface temperature	108.87	4.87	0.029	0.099
Watershed size + Width	108.9	4.9	0.028	0.111

Table 3.5. Reach-scale habitat measurements. Substrate temperature, depth and riparian canopy cover were used as scale-specific independent variables in the reach-scale models.

Reach- scale habitat variables	Mean	Min – Max	Standard Error
Depth (mm)	210.6	68.6 – 759.3	±162.0
Substrate temperature (°C)	14.7	10.3 – 18.9	±2.90
Riparian canopy cover (%)	35.6	1 - 99	±28.6
Surface temperature (°C)	15.4	11.0 – 19.9	±2.96
Stream width (m)	3.2	1.04 – 6.83	±2.16
Stream discharge (m^3/s)	0.031	0.002 – 0.101	±0.00185

Table 3.6. Top weighted AIC models explaining the heterogeneity in reach-scale estimated Brook Trout abundance.

Model variables	AICc	Delta AICc	AICc weight	Adjusted R^2
Canopy Cover	52.93	0.00	0.277	0.068
Canopy Cover + Watershed size	53.63	0.69	0.196	0.143
Watershed size	54.56	1.62	0.123	-0.02
Depth (mm)	55.20	2.27	0.089	-0.058
Substrate Temperature	55.27	2.34	0.086	-0.062
Canopy Cover + substrate temperature	55.55	2.62	0.075	0.046
Canopy cover + Depth	55.67	2.72	0.071	0.040
Canopy Cover + Watershed size + Depth	57.46	4.53	0.029	0.086
Canopy Cover + Watershed size + Substrate Temperature	57.54	4.61	0.028	0.082
Watershed size + substrate temperature	57.60	4.67	0.027	-0.069

Table 3.7. Microhabitat- scale habitat measurements. Substrate temperature, depth and riparian canopy cover were used as the scale-specific independent variables in the microhabitat-scale models.

Microhabitat-scale habitat variables	Mean	Min – Max	Standard Error
Substrate temperature (°C)	15.4	6.2 – 20.97	± 3.4
Depth (mm)	287.5	61 - 645	± 176
Riparian canopy cover (%)	47	0 - 100	± 33.5
Surface Temperature (°C)	16.3	6.5 – 21.3	± 3.1
Stream Width (m)	3.2	0.92 – 6.83	± 1.9
Stream Discharge (m^3/s)	0.035	0.0009 – 0.10	± 0.035

Table 3.8. Top weighted AIC models explaining the heterogeneity in microhabitat-scale Brook Trout seconds.

Model Variables	AICc	Delta AICc	AICc weight	Adjusted R
Surface temperature + Discharge	252.77	0.00	0.391	0.134
Surface temperature + Width	253.84	1.06	0.23	0.12
Surface temperature + Width + Discharge	255.11	2.33	0.122	0.12
Width	256.1	3.32	0.074	0.071
Surface temperature + Width + Depth	256.16	3.38	0.072	0.106
Surface temperature + Width + Discharge + Cover	257.42	4.6	0.039	0.107
Depth + Width	258.28	5.5	0.025	0.057
Surface temperature + Cover + Depth + Width	258.42	5.6	0.023	0.092
Surface temperature + Discharge + Depth + Width + Watershed size	259.75	7.0	0.012	0.093
Cover + Discharge + Width	259.83	7.1	0.012	0.053

Chapter 3 Figures

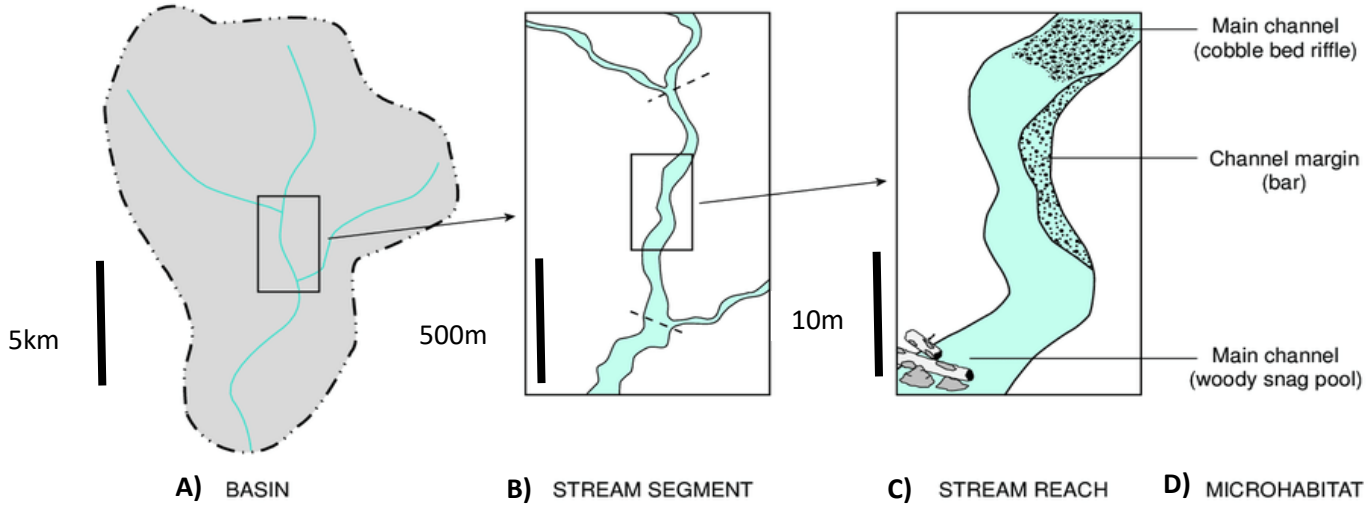

Figure 3.1. Spatial hierarchy of a A) watershed basin, B) stream segment, C) stream reach and D) microhabitat. From Fitzpatrick et al. (1998).

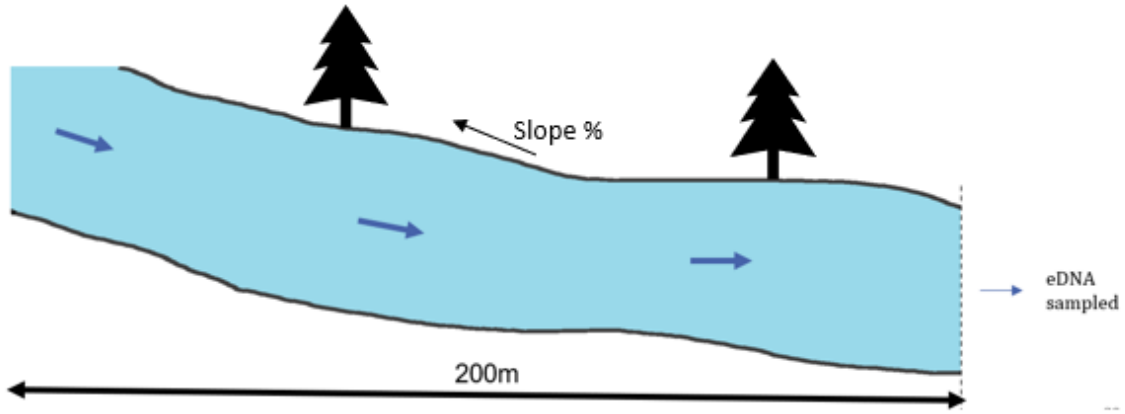

Figure 3.2. Visual representation of the segment-scale habitat variables measured. Forested land cover, slope and baseflow index were generated using the Ontario Flow Assessment Tool (OFAT, 2013).

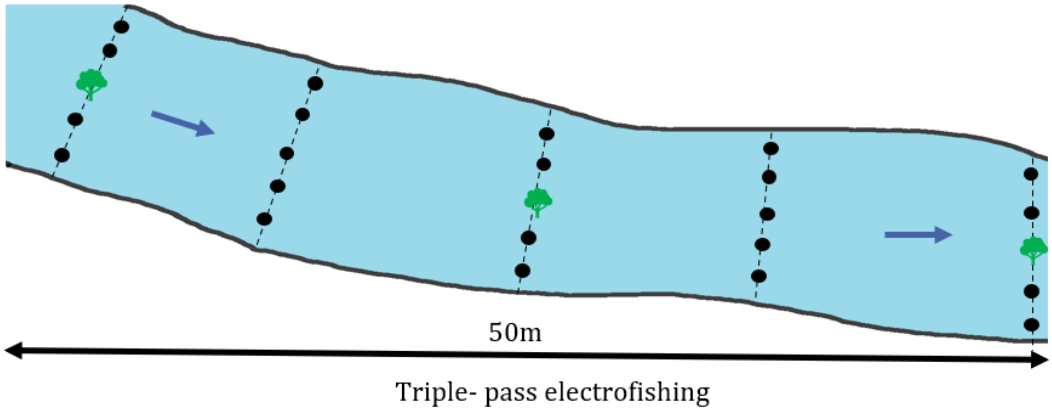

Figure 3.3. Visual representation of the reach-scale habitat variables measured. Substrate temperature and depth measurements were taken at each black dot and averaged over the stream reach while canopy cover was taken at three green trees and averaged over the stream reach.

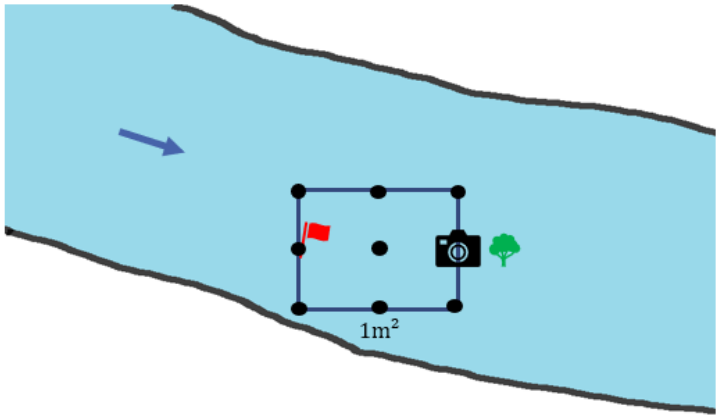

Figure 3.4. Visual representation of the microhabitat-scale habitat variables measured. Substrate temperature was taken at each black dot and averaged over the microhabitat. Depth measurements were taken at the camera, at the midpoint between the camera and the flag and at the flag and averaged over the three measurements. Canopy cover was taken at the camera location.

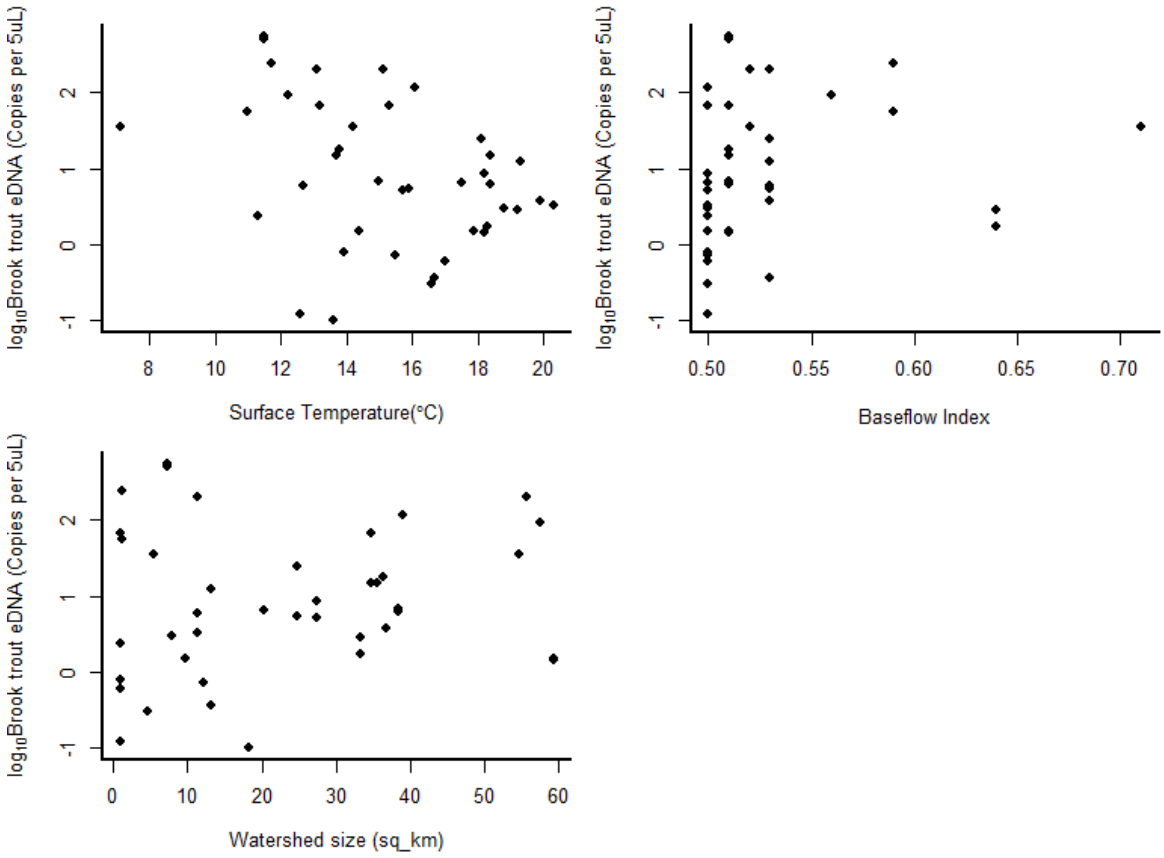

Figure 3.5. Habitat-associations with Brook Trout eDNA at the segment-scale. The top AICc model included the variables: surface water temperature, baseflow index and watershed size; Table 3.4; AICc = 103.99, r^2= 0.187).

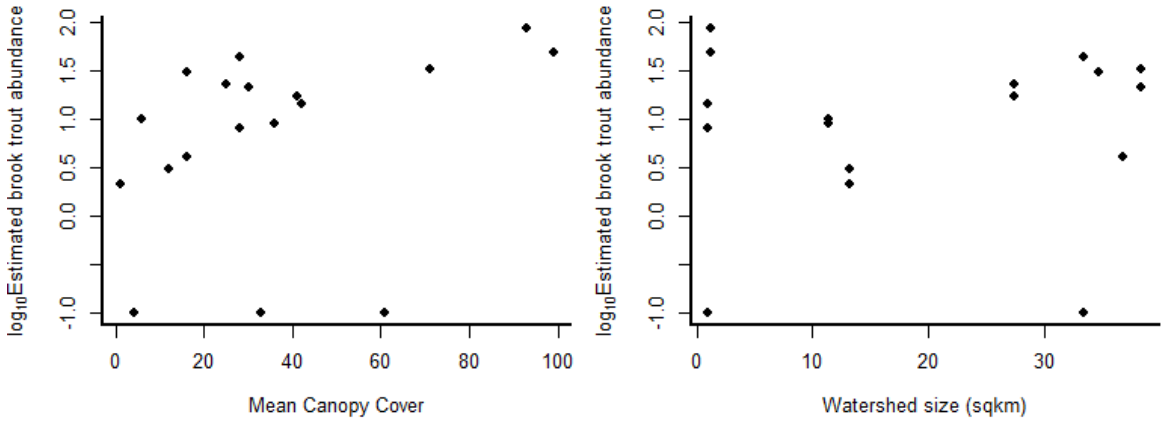

Figure 3.6. Habitat associations with estimated Brook Trout abundance at the reach scale. The top AICc model included the variables: canopy cover and watershed size (Table 3.6; AICc = 53.63, r^2=0.143).

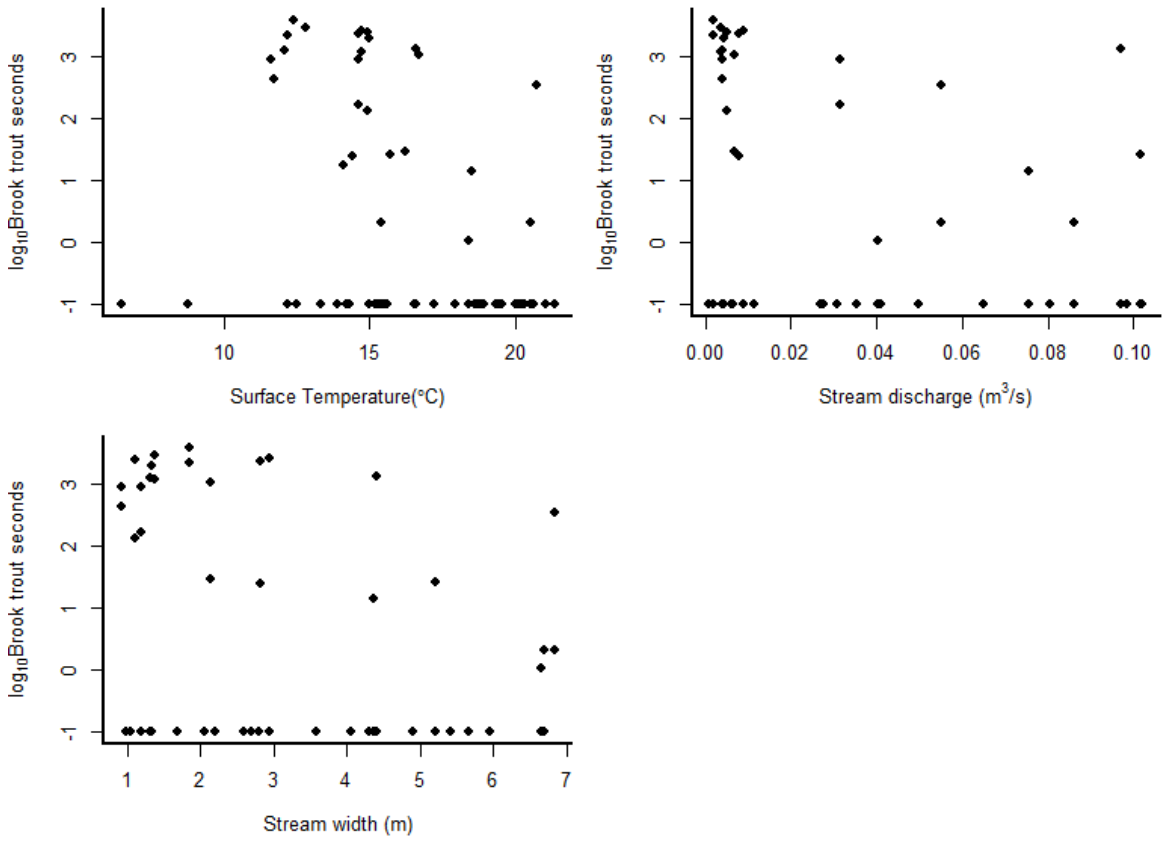

Figure 3.7. Habitat associations with Brook Trout seconds at the microhabitat scale. The top AICc model included the common habitat variables: surface temperature, stream discharge and stream width (Table 3.8; AICc = 252.77, r^2=0.134).

Chapter 4. General Conclusions

Results from this study found a strong association between estimates of Brook Trout abundance obtained through triple-pass electrofishing and environmental DNA levels in both 2019 and 2020, supporting the use of eDNA techniques as an alternative method for determining Brook Trout presence/absence and abundance estimates in stream environments. Brook Trout abundance estimates obtained from underwater videos also showed a strong correlation with estimates of Brook Trout abundance obtained through conventional (electrofishing) methods. Environmental DNA concentrations exhibited a stronger agreement with Brook Trout abundance estimates from electrofishing than UWVC surveys, suggesting that eDNA may be better suited as a potential alternative sampling method, particularly at large spatial scales. Although the agreement with UWVC was not as strong, relative to electrofishing, both these methods reduce the possibility of stress, harm and potential mortality to Brook Trout. However, the causes of interannual variation between sampling years needs to be accounted for in order to use these methods as predictive tools for Brook Trout abundance.

An advantage of using both eDNA and UWVC methods is that they are relatively simple to deploy, require minimal field equipment, only require a crew of 2 and are much less labor-intensive. Both methods require some level of laboratory and/or visual analysis but the advantage of eDNA and UWVC is how much faster it is to obtain field samples. However, the processing time for underwater videos can be considerable if fish are constantly moving in and out of the camera's field of view. For example, a video that has several dace species and Brook Trout swimming in and out of the frame could take up to 2 hours per microhabitat for analysis. As I had 68 microhabitat samples, approximately 136

hours of video watching went into this study. Development of automated analyses using AI programs that can identify different species of fish could reduce the time and effort required. Although qPCR analysis took place at Trent University, it can be speculated that the qPCR processing took considerable time which may offset the efficiencies gained in the field but the overall processing time for eDNA filtering at CNFER took approximately 1 hour per stream segment, which includes the time it takes to clean and disinfect all the equipment. Electrofishing a single reach typically requires a minimum crew of 3 and takes approximately 10 hours, which includes three passes, processing captured fish, releasing fish, packing up gear, transporting gear back to the vehicle and driving back to the lab. The relative portability and limited equipment required for UWVC and eDNA surveys also makes these tools inherently valuable for surveying stream sections in areas that are difficult to get to with more cumbersome equipment such as electrofishers.

Variation in eDNA concentrations, underwater video abundance and Brook Trout abundance estimates existed across sites and sampling years. This is not surprising as reaches experience fluctuations in stream temperatures, flows and depths annually, but the higher eDNA levels in 2020 coupled with a lower relative abundance of Brook Trout and lower MaxN counts and seconds in 2020 was surprising. In theory, higher eDNA levels should correlate with higher Brook Trout abundance estimates. Variation in abundance estimates may have been caused from environmental conditions experienced in 2020. Streams in 2020 had higher average substrate and surface water temperatures and were shallower, which may have increased the metabolic rate of individual Brook Trout leading to increased rates of shedding epidermal cells or other secretions (Wilcox et al., 2016; Rourke et al., 2021). Further, the increase in water temperatures may have caused Brook

Trout to seek thermal refugia in microhabitats and caused Brook Trout to become "clumped" in areas that were not within the microhabitats sampled.

To make confident conclusions about eDNA levels as they relate to Brook Trout abundance, the environmental conditions where the sample is taken need to be considered. Environmental conditions influence Brook Trout life-history attributes, which will ultimately influence the amount of DNA shed from individual Brook Trout. Some reaches had very high eDNA concentrations (>400 copies per 5 uL) which may have been attributed to environmental conditions. The high eDNA levels were found in streams with relatively colder substrate temperatures which may have influenced Brook Trout activity levels, and number of Brook Trout in the stream or even reduced eDNA degradation. Repeated samples of eDNA, during a sampling event and over the season, at all reaches and measuring habitat conditions would be valuable for explaining why high eDNA levels were found compared to other sites.

This study showed that alternative methods can be used to determine Brook Trout presence (and potentially abundance) and that these methods can be used to determine associations between habitat characteristics and Brook Trout abundance and distribution at different spatial scales. As Brook Trout are generally associated with a suite of habitat characteristics (e.g., cold stream temperatures, groundwater inputs, canopy cover), some of these characteristics may be more strongly associated with Brook Trout abundance at different spatial scales while some characteristics may be important at all scales. Cold water temperatures emerged as an important variable at both the segment and microhabitat scale and could also be important at the reach scale which is part of the larger and smaller spatial units. Thus, if we know that cold water temperatures are important at all spatial

scales, then fisheries managers should implement actions that preserve cold water temperatures broadly. In contrast, if other types of habitat features emerge as important at certain spatial scales, then those features may be more important for considering certain attributes of Brook Trout life history and could be implemented differently based on priority/ accessibility to streams. Therefore, resource managers should prioritize habitat managements actions based on ones that can affect change on (e.g., climate cannot be changed but increasing riparian canopy cover can) and ultimately determine where conservation efforts should be implemented.

As Brook Trout continue to be adversely affected by anthropogenic impacts and climate change in their native stream ranges, a rapid tool that can evaluate Brook Trout populations and their associations with habitat features while avoiding potential injury, habitat destruction and even mortality, is valuable for fisheries managers. Brook Trout in Lake Superior tributaries have not experienced that same level of anthropogenic disturbance as have southern populations, but many landscape alterations (e.g., forestry and mining) still threaten Brook Trout populations in the north. Further, the effects of climate change are substantial to species that rely on the availability of cold-water habitats. Future projections of Brook Trout populations show them shifting their populations to higher elevation streams that provide an ample supply of cold-water (Chu et al., 2005). Using alternative sampling tools, coupled with the knowledge of Brook Trout habitat-associations can aid in predicting Brook Trout habitat suitability and distribution across multiple spatial scales.

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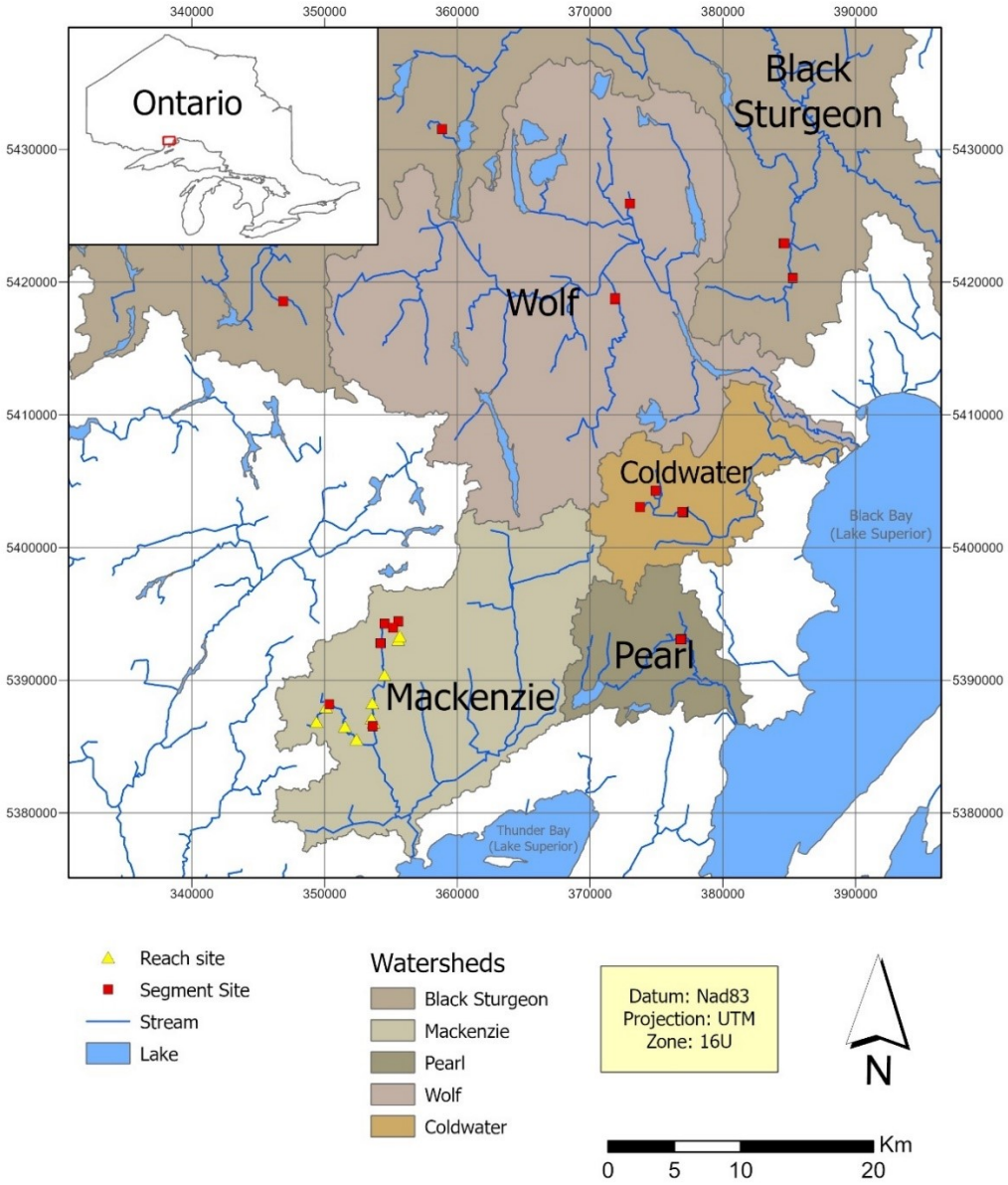
Appendix A



A1- Map of the watersheds along the north shore of Lake Superior. Surveys were conducted within the Black Sturgeon, Coldwater, Pearl, Mackenzie and Wolf watersheds. The red points denote the stream segments, and the yellow points denote the reaches where electrofishing surveys occurred.

A2- Raw data table for reach scale habitat characteristics and estimated Brook Trout abundance

Site	Date	Estimated Abundance	Depth	Substrate Temp	Canopy Cover	Wetted Width	Surface Temp	Discharge	Watershed Size (km^2)
EW1K	2019	86	83.3	10.7	93	1.85	11	0.0018	1.2
Walk10	2019	44	156	18.13	28	6.83	18.3	0.0549	33.4
EW_20	2019	32	186.7	18.17	71	6.66	15	0.0402	38.4
EW_17	2019	17	759.33	10.3	41	5.22	15.7	0.1017	27.4
Walk6	2019	9	116.33	12.9	36	1.37	12.7	0.0036	11.4
YoungIn_Below	2019	8	103.33	11.55	28	1.33	11.3	0.0043	1
Dsouth	2019	3	322.33	16.37	12	2.06	16.7	0.0308	13.2
YoungIn_Above	2019	0	82	12.56	61	1.19	12.6	0.0018	1
EW1K	2020	49	68.6	11	99	1.32	11.7	0.0041	1.2
160719	2020	30	139.48	18.4	16	2.8	15.3	0.0269	34.7
EW_17	2020	23	369.52	18.9	25	4.4	18.2	0.0969	27.4
EW_20	2020	21	204.28	15.7	30	6.7	18.4	0.0861	38.4
YoungIn_Below	2020	14	103.6	14.5	42	1.2	13.2	0.0044	1
Walk6	2020	10	208.4	13.5	6	2.15	15.1	0.0067	11.4
Wwalk_S1L	2020	4	290.4	18.1	16	5.95	19.9	0.0314	36.8
Dsouth	2020	2	250.08	13.6	1	2.2	19.3	0.0276	13.2
YoungIn_Above	2020	0	134.7	16	33	1.04	13.9	0.0066	1
Walk10	2020	0	212.6	14.5	4	3.58	19.2	0.0272	33.4

A3- Raw data table for segment scale habitat characteristics and Brook Trout eDNA concentrations

Site	Date	eDNA (copies per 5uL)	Stream width	Discharge	Surface Temp	Watershed Size	Slope (%)	Landcover (%)
Walk10	2019	1.64	6.83	0.0549	18.3	33.4	0.64	90.6
EW_20	2020	5.99	6.7	0.0861	18.4	38.4	1.06	86
EW_20	2019	6.74	6.66	0.0402	15	38.4	1.06	86
Wwalk_S1L	2020	3.58	5.95	0.0314	19.9	36.8	1.03	77.1
SwardBigP_50K_L	2020	1.41	5.66	0.0804	17.9	59.3	0.9	72.3
Pine_10K	2020	3.15	5.42	0.0649	20.3	11.4	1.6	51.3
EW_17	2019	5.05	5.22	0.1017	15.7	27.4	0.31	82.3
SwardBigP_50K_S	2020	1.34	4.9	0.1020	18.2	59.3	0.9	72.3
EW_17	2020	8.36	4.4	0.0969	18.2	27.4	0.31	82.3
EW_3	2020	113.95	4.36	0.0757	16.1	39.1	0.8	58.5
160719	2019	15.05	4.3	0.0499	18.4	34.7	1.06	84.5
DnorBridge	2019	5.24	4.15	0.0617	15.9	24.8	0.79	82.7
Nicholson_50K_L	2020	90.92	4.05	0.0984	12.2	57.5	0.5	76.2
150719	2019	6.47	3.7	0.0221	17.5	20.3	0.95	56.8
Walk10	2020	2.78	3.58	0.0411	19.2	33.4	0.64	90.6
Fur_1S	2020	35.33	2.94	0.0089	14.2	54.7	0.52	54.04
Fur_1L	2020	203.86	2.82	0.0079	13.1	55.7	0.52	54.04
160719	2020	66.24	2.8	0.0269	15.3	34.7	1.06	84.5
170719	2019	0.2	2.7	0.0134	16.6	4.6	1.6	41.6
DnorBridge	2020	24.38	2.69	0.0354	18.1	24.8	0.79	82.7
SamAs_20K_L	2020	0	2.6	0.0114	13.6	18.2	0.52	79.6
Dsouth	2020	12.02	2.2	0.0276	19.3	13.2	0.94	86.1
Walk6	2020	199.74	2.15	0.0067	15.1	11.4	1.18	87.5
Dsouth	2019	0.27	2.06	0.0308	16.7	13.2	0.94	86.1
EW1K	2019	55.95	1.85	0.0018	11	1.2	4.2	98.1
Walk4.2	2019	2.9	1.75	0.0117	18.8	7.9	0.97	53.7
Seagull_10K_L	2020	0.64	1.68	0.0060	15.5	12.2	1.3	77
Walk6	2019	5.84	1.37	0.0036	12.7	11.4	1.18	87.5
YoungIn_Below	2019	2.33	1.33	0.0043	11.3	1	0	99.5
EW1K	2020	238.24	1.32	0.0041	11.7	1.2	4.2	98.1
YoungIn_Below	2020	67.32	1.2	0.0044	13.2	1	0	89.5
YoungIn_Above	2019	0.02	1.19	0.0018	12.6	1	0	99.5
Driftstone_L	2020	553.84	1.12	0.0050	11.5	7.2	1.09	57.1
YoungIn_Above	2020	0.69	1.04	0.0066	13.9	1	0	99.5
Beaverhide_14K	2020	35.39	0.99	0.0009	7.1	5.4	2.9	57.5
Driftstone_S	2020	513.6	0.92	0.004	11.5	7.2	1.08	57.1
Dam1K	2019	0.49	0.45	0.0078	17	0.9	1.8	30.6
Ouimet_10_2	2020	1.38	-	0.0199	14.4	9.7	0.9	70.7

A3- Raw data table for segment scale habitat characteristics and Brook Trout eDNA concentrations continued

Site	Date	eDNA (copies per 5uL)	Stream width	Discharge	Surface Temp	Watershed Size	Slope (%)	Landcover (%)
EscQui2Wf	2020	17.77	-	-	13.8	36.3	2.34	65.3
EscQuiBx	2020	14.47	-	-	13.7	35.6	2.34	65.3

A4- Raw data tables for microhabitat scale habitat characteristics and Brook Trout MaxN counts and seconds.

Site	Date	Count	Time	Location	Substrate Temp	Surface Temp	Depth	Canopy cover	Wetted Width	Discharge	Watershed Size
160719	2019	0	0	cold	19.28	19.3	110	52.5	4.3	0.0499	34.7
160719	2019	0	0	cold	19.44	19.5	255	72.5	4.3	0.0499	34.7
Dsouth	2019	0	0	cold	17.03	19.4	150	12.25	2.06	0.0308	13.2
Dsouth	2019	0	0	cold	15.95	19.5	508	11.5	2.06	0.0308	13.2
EW_17	2019	1	27	cold	7.87	15.7	845	50	5.22	0.1017	27.4
EW_17	2019	0	0	cold	8.68	15.5	858	32.5	5.22	0.1017	27.4
EW_20	2019	1	1	cold	18.26	18.4	305	58.75	6.66	0.0402	38.4
EW_20	2019	0	0	cold	17.95	18.4	610	82.5	6.66	0.0402	38.4
EW1K	2019	3	3948	cold	12.31	12.4	67	88.75	1.85	0.0018	1.2
EW1K	2019	2	2191	cold	11.79	12.2	125	73	1.85	0.0018	1.2
Walk10	2019	1	2	cold	19.65	20.5	220	21.5	6.83	0.0549	33.4
Walk10	2019	1	352	cold	19.74	20.7	235	34	6.83	0.0549	33.4
Walk6	2019	3	2975	cold	12.97	12.8	171	36.5	1.37	0.0036	11.4
Walk6	2019	2	1178	cold	13.39	14.7	278	35.5	1.37	0.0036	11.4
YoungIn_ Above	2019	0	0	cold	13.04	13.3	116	70	1.19	0.0018	1
YoungIn_ Above	2019	0	0	cold	13.14	13.9	119	52.75	1.19	0.0018	1
YoungIn_ Below	2019	0	0	cold	14.18	15.2	178	39.25	1.33	0.0043	1
YoungIn_ Below	2019	1	1964	cold	14.44	15	192	16.75	1.33	0.0043	1
160719	2020	0	0	cold	16.49	16.5	185	32.5	2.8	0.0269	34.7
160719	2020	0	0	cold	16.64	16.6	210	10	2.8	0.0269	34.7
Dsouth	2020	0	0	cold	19.6	20	225	0	2.2	0.0276	13.2
Dsouth	2020	0	0	cold	19.11	20.1	501	0	2.2	0.0276	13.2
EW_17	2020	0	0	cold	16.13	16.5	185	72.5	4.4	0.0969	27.4
EW_17	2020	1	1332	cold	16.49	16.6	210	48.75	4.4	0.0969	27.4
EW_20	2020	1	2	cold	15.43	15.4	165	62.5	6.7	0.0861	38.4
EW_20	2020	0	0	cold	15.79	15.5	213	56.25	6.7	0.0861	38.4
EW1K	2020	1	1260	cold	11.98	12.1	61	68.75	1.32	0.0041	1.2
EW1K	2020	0	0	cold	12.46	12.5	100	100	1.32	0.0041	1.2
Walk10	2020	0	0	cold	20.97	21	299	37.5	3.58	0.0411	33.4
Walk10	2020	0	0	cold	20.38	20.5	333	52.5	3.58	0.0411	33.4
Walk6	2020	1	1083	cold	14.5	16.7	478	5	2.15	0.0067	11.4
Walk6	2020	1	29	cold	14.52	16.2	531	4.5	2.15	0.0067	11.4
Wwalk_S1L	2020	0	0	cold	20.09	20.2	361	70	5.95	0.0066	36.8
Wwalk_S1L	2020	0	0	cold	20.42	20.6	412	68.75	5.95	0.0066	36.8
YoungIn_ Above	2020	0	0	warm	15.2	15.5	136	25	1.04	0.0044	1
YoungIn_ Above	2020	0	0	warm	14.7	15.4	201	17.5	1.04	0.0044	1
YoungIn_ Below	2020	1	173	warm	14.21	14.6	151	17.5	1.2	0.0314	1
YoungIn_ Below	2020	3	911	warm	14.4	14.6	197	3.75	1.2	0.0314	1
EscQui2Wf	2020	0	0	warm	15.3	15.3	146.5	0			36.3

A4- Raw data tables for microhabitat scale habitat characteristics and Brook Trout MaxN counts and seconds continued

Site	Date	Count	Time	Location	Substrate Temp	Surface Temp	Depth	Canopy cover	Wetted Width	Discharge	Watershed Size
EscQui2Wf	2020	0	0	warm	14.9	15	214.8	0			36.3
EscQuiBx	2020	0	0	warm	14.2	14.2	300	40			35.6
EscQuiBx	2020	1	18	warm	14.2	14.1	438	43			35.6
SwardBigP_50K_S	2020	0	0	warm	18.8	18.7	424.5	69	4.9	0.102	59.3
SwardBigP_50K_S	2020	0	0	warm	18.8	18.8	656	93	4.9	0.102	59.3
SwardBigP_50K_L	2020	0	0	warm	18.4	18.9	212.5	3	5.66	0.0804	59.3
SwardBigP_50K_L	2020	0	0	warm	18.4	18.6	644.5	31	5.66	0.0804	59.3
Pine_10K	2020	0	0	warm	20.8	21.3	315	0	5.42	0.0649	11.4
Pine_10K	2020	0	0	warm	20.3	21.3	584.5	0	5.42	0.0649	11.4
Nicholson_50K_L	2020	0	0	warm	12.4	12.2	523.5	74	4.05	0.0984	57.5
Nicholson_50K_L	2020	0	0	warm	12.6	15.6	474	100	4.05	0.0984	57.5
Driftstone_L	2020	1	134	warm	11.7	14.9	219.5	100	1.12	0.005	7.2
Driftstone_L	2020	1	2575	warm	11.6	14.9	196.5	100	1.12	0.005	7.2
Driftstone_S	2020	2	908	warm	11.6	11.6	234.5	100	0.92	0.004	7.2
Driftstone_S	2020	2	428	warm	11.7	11.7	162	100	0.92	0.004	7.2
Seagull_10K_L	2020	0	0	warm	14.5	17.9	348.5	100	1.68	0.006	12.2
Seagull_10K_L	2020	0	0	warm	15.5	17.2	130	100	1.68	0.006	12.2
SamAs_20K_L	2020	0	0	warm	13.8	14.3	144.5	60	2.6	0.0114	18.2
SamAs_20K_L	2020	0	0	warm	13.9	14.3	257.5	36	2.6	0.0114	18.2
Beaverhide_14K	2020	0	0	warm	7.9	8.8	143	100	0.99	0.0009	5.4
Beaverhide_14K	2020	0	0	warm	6.2	6.5	184.5	0	0.99	0.0009	5.4
Fur_1L	2020	1	25	warm	14.2	14.4	239	8	2.82	0.0079	55.7
Fur_1L	2020	2	2325	warm	14.4	14.6	290.5	11	2.82	0.0079	55.7
Fur_1S	2020	3	2602	warm	14.5	14.7	461	36	2.94	0.0089	54.7
Fur_1S	2020	0	0	warm	15.1	15	161.5	8	2.94	0.0089	54.7
EW_3	2020	1	14	warm	17.9	18.5	225	91	4.36	0.0757	39.1
EW_3	2020	0	0	warm	17.8	18.8	254.5	39	4.36	0.0757	39.1
DnorBridge	2020	0	0	warm	19.8	20	174	65	2.69	0.0354	24.8
DnorBridge	2020	0	0	warm	19.2	20.3	290.5	64	2.69	0.0354	24.8